THE EFFECT OF INCREASED TEMPERATURE AND PCO_2 ON RESPIRATION AND PHOTOSYNTHETIC RATES IN THE RED ALGA *Gracilaria salicornia*

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Table of Contents

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Abstract

Increasing temperatures and ocean acidification are causing widespread damage to coral reefs. Since corals and macroalgae compete for resources, this may lead to the proliferation of some macroalgal species. Understanding how macroalgae will be affected by climate change will be useful knowledge for conservation management. This study focuses on how the interactive factors of temperature and pCO_2 affect the respiration and photosynthesis rates of *Gracilaria salicornia*, an invasive alga in Hawai‘i originally from the eastern Pacific. Using an outdoor, continuous-flow seawater system with natural sunlight, *G. salicornia* (n=8) tissue was exposed to different temperatures and pCO_2 levels using a full-factorial design. At the end of a three-day exposure to treatment conditions, respiration was measured using oxygen evolution methods and photosynthesis was measured using a fluorometer to obtain rapid light curves. Each response variable was analyzed independently using multiple regression models. The models suggest temperature and temperature^2 had significant effects on respiration rates ($P<0.03$ and $P<0.03$, respectively). Analysis indicates pCO_2 had a slight but significant effect on maximum electron transfer rate (ETR_{max}) ($P<0.04$). Results indicate *G. salicornia* experiences carbon limitation, which has also been demonstrated in other species of the genus *Gracilaria*. The increase in carbon availability that accompanies ocean acidification may encourage growth in these carbon-limited species. These analyses show an increase in respiration and photosynthesis rates under select climate change scenarios, though further research will be needed to determine how these metabolic increases affect the growth and distribution of the organism.

List of Tables

List of Figures

List of Abbreviations

AFRC – Ānuenue Fisheries Research Center

CA – carbonic anhydrase

DIC – dissolved inorganic carbon

DO – dissolved oxygen concentration

E_k – minimum saturating irradiance (used with fluorometry methods)

ETR_{max} – maximum electron transport rate (used with fluorometry methods)

HIMB – Hawai‘i Institute of Marine Biology

I_k – minimum saturating irradiance (used with dissolved oxygen methods)

PAR – photosynthetically active radiation

pCO_2 – the partial pressure exerted by carbon dioxide gas in the air above a liquid

P_{max} – maximum photosynthesis rate (used with dissolved oxygen methods)

TA – total alkalinity

Chapter 1: Literature Review

Global climate change and coral reefs

Over the next century, the amount of carbon dioxide (CO_2) gas in earth's atmosphere is projected to increase with anthropogenic input (IPCC, 2014). By 2100, atmospheric CO_2 levels are predicted to reach 940 µatm, greater than a three-fold increase from pre-industrial levels of 280 µatm (Hoegh-Guldberg *et al.*, 2007; Pörtner *et al.*, 2014). Carbon dioxide is a greenhouse gas, trapping and reradiating heat within the atmosphere, warming the planet (Hoegh-Guldberg *et al.*, 2007; Pörtner *et al.*, 2014). Much of this increase in CO_2 will be distributed by winds and diffuse into ocean waters, changing the chemistry of seawater and lowering the pH through a process known as ocean acidification. Ocean acidification will increase the absolute amount of CO_2, which in turn will cause changes in concentrations of different carbon compounds as seawater chemistry attains equilibrium (Hoegh-Guldberg *et al.*, 2007). These changes in chemistry will affect many different life processes for diverse marine organisms, including calcification, membrane transport, photosynthesis, and reproductive success (Hoegh-Guldberg *et al.*, 2007; Pörtner *et al.*, 2014).

Future climate change conditions, such as decreased ocean pH and warmer ocean water temperatures, will disrupt coral reef ecosystems (Hoegh-Guldberg *et al.*, 2007; Hughes *et al.* 2007; Koch *et al.*, 2013; Kroeker *et al.*, 2013; Pörtner *et al.*, 2014). Corals, which provide the foundation for many tropical coastal ecosystems, are sensitive to these changes and are already beginning to face major challenges as a result (Jokiel & Brown, 2004; Hoegh-Guldberg *et al.*, 2007; Koch *et al.*, 2013; Kroeker, Micheli, & Gambi, 2013; Pörtner *et al.*, 2014; Bahr, Jokiel & Rodgers, 2015; Rodgers *et al.*, 2017). Within the past three years, widespread coral bleaching *events* - the loss of the algal symbiont under stressful conditions - have been reported in Hawai'i

(Bahr, Jokiel & Rodgers, 2015; Rodgers *et al.*, 2017), the Great Barrier Reef (Hughes *et al.*, 2017), Taiwan (DeCarlo *et al.*, 2017), and Indonesia (Ampou *et al.*, 2017). Coral bleaching occurs as a stress response to high temperatures, and in severe, long-lasting cases, coral bleaching results in coral mortality (Hoegh-Guldberg, 1999; Wilkinson *et al.*, 1999; Jokiel & Brown, 2004). In addition to rising temperatures, corals are facing a more acidic seawater environment, which inhibits their ability to calcify (Hoegh-Guldberg *et al.*, 2007; Pörtner *et al.*, 2014). Increased pCO_2 has distinct effects on the coral organism at different life stages, although there is contrasting evidence regarding the relative vulnerabilities of coral larvae, juveniles, and adult colonies to increased pCO_2 (Anlauf, D'Croz & O'Dea, 2011; Kroeker *et al.*, 2013; Kroeker, Micheli & Gambi, 2013; Przeslawski, Byrne & Mellin, 2015). Coral species demonstrate varying levels of vulnerability to these conditions (Bahr, Jokiel & Rodgers, 2016; Okazaki *et al.*, 2017), and long-term exposure to higher temperatures and ocean acidification can change community structure and reduce diversity (Hoegh-Guldberg *et al.*, 2007; Kroeker, Gambi & Micheli, 2013; Huang & Roy, 2015).

Climate change will alter many parts of the coral ecosystem. The reaction of populations to these stressors can be summarized as follows: (1) if motile, organisms can move to new areas, (2) they can acclimate to the new conditions, (3) they can adapt genetically over multiple generations, (4) they can die, resulting in extirpation or extinction (Harley *et al.*, 2012; Hoegh-Guldberg *et al.*, 2017). For example, among reef fishes, the species that have small ranges and are highly dependent on coral are expected to decline (Munday *et al.* 2008). Like other mobile species, reef fishes will probably shift their range based on preferred temperature gradients (Munday *et al.* 2008). Crustose coralline algae and calcifying macroalgae show decreased photosynthetic and growth rates in ocean acidification treatments (Koch *et al.*, 2013; Kroeker *et*

al., 2013; Johnson, Price & Smith, 2014). In general, taxa more vulnerable to climate change conditions, including coralline algae, echinoderms, and mollusks, will likely decrease in abundance while organisms with greater resilience, like crustaceans, fleshy macroalgae, and sea grasses, increase in abundance (Kroeker *et al.*, 2013). The varying levels of vulnerability to climate change can result in changes to community structure or in more extreme cases, extirpations and extinctions (Hoegh-Guldberg *et al.*, 2007; Kroeker *et al.*, 2013; Kroeker, Gambi & Micheli, 2013; Kroeker, Micheli & Gambi, 2013; Pörtner *et al.*, 2014; Huang & Roy, 2015).

Studies examining climate change in the marine environment have generally focused on corals and other calcifying invertebrates, but other important taxa have been relatively overlooked. Over 70% of marine climate change papers published between 2000-2009 examined the effect of climate change on benthic invertebrates (Wernberg, Smale & Thomas, 2012). The importance of coral research cannot be denied; however, it is also essential to examine the impact that climate change will have on other organisms, especially those that heavily influence the condition of coral reef ecosystems. Foremost among these are macroalgae, a major competitor with corals and a provider of food and oxygen to the reef, yet only 11% of publications looked at the effects of climate change on these important primary producers (Wernberg, Smale & Thomas, 2012; Koch *et al.*, 2013). Macroalgal research is necessary to more fully understand how climate change will affect the coral reef ecosystem as a whole.

Invasive algae in Hawai‘i

At least 19 species of foreign marine macroalgae have been transported purposefully or accidentally to the Hawaiian Islands since the 1950s (Russell, 1992; Smith, Hunter & Smith, 2002). Many were purposefully introduced as agar or carrageenan crops for commercial purposes, while others are thought to be accidental introductions, arriving on ship hulls, in ballast

materials, as epiphytes on imported aquaculture, or as aquarium releases (Russell, 1992; Smith, Hunter & Smith, 2002; Godwin, Rodgers & Jokiel, 2006). Some of these species have become invasive- growing and spreading over large areas in a relatively short period of time.

Invasive algal blooms have financial and ecological repercussions (Russell, 1992; Cesar *et al.* 2002; Cesar & van Beukering, 2004; Godwin, Rodgers & Jokiel, 2006). For example, many tons of rotting algae strewn along Maui beachfront property from overgrowth of the introduced and invasive *Hypnea musciformis* is estimated to have cost the City and County of Maui over $20 million dollars from lowered property values, decreases in rental income, and cleanup costs (Cesar *et al.*, 2002). Introduced and invasive algae have the potential to compete successfully for ecological niches formerly occupied by native algae (Russell, 1992). Russell demonstrated how two introduced and invasive algae in Hawai‘i (*Hypnea musciformis* and *Acanthophora spicifera*) are dominant in the competition with two native algae (*Hypnea cervicornis* and *Laurencia nidifica*) that occupy similar niches (1992). Algal mats or mounds can alter the benthic environment. For example, the invasive, mound-forming *Gracilaria salicornia* can create environments of hypoxia, hyperoxia, acidification, increased sedimentation, and decreased irradiance beneath the algal mound structure (Martinez, Smith & Richmond, 2012). These conditions cause stress to corals and other benthic organisms living underneath (Martinez, 2012). Invasive algae harbor different communities of epiphytes and epifauna than native algae (Fukunaga, Peyton & Thomas, 2014). Research from Hawai‘i indicates invasive algae cause changes in community composition, affect higher trophic levels, change habitats, and monopolize spatial resources (reviewed in Davidson *et al.*, 2015).

An important factor controlling invasive algal blooms in Hawai‘i is nutrient abundance. Increasing the nitrogen or phosphorus nutrient availability significantly increases the growth of

Hawai‘i’s marine algae, and some invasive algae are able to sequester nutrients within microenvironments created from the 3-D structure of their thalli (Stimson, Larned & McDermid, 1996; Larned, 1998). Eutrophic environments associated with wastewater injection wells and submarine groundwater discharge encourage the growth of invasive algae (Dailer, Smith & Smith, 2012). The continued discharge of sewage over several decades into Kāne‘ohe Bay has been linked to the overgrowth of the native, invasive macroalga *Dictyosphaeria cavernosa*, and the subsequent diversion of sewage outfall to deeper ocean waters caused immediate decrease in the standing crop of *D. cavernosa* within the bay (Smith *et al.*, 1981; Hunter & Evans, 1995).

The most successful invasive algae in Hawai‘i are *Avrainvillea amadelpha* (Chlorophyta) and rhodophytes *Eucheuma denticulatum and Kappaphycus alvarezii*, *Hypnea musciformis*, *Acanthophora spicifera*, and *Gracilaria salicornia* (Smith, Hunter & Smith, 2002; Godwin, Rodgers & Jokiel, 2006). *Gracilaria salicornia* was chosen as the focus of this research because of its ecological significance in Hawai‘i as an invasive alga.

Gracilaria salicornia

Gracilaria salicornia (C. Agardh) Dawson is a highly invasive alga in Hawai‘i that covers large swaths of shallow coastal areas around O‘ahu, Hawai‘i Island, and Moloka‘i (Rodgers & Cox, 1999; Nelson *et al.*, 2009). In its native range, *G. salicornia* is widely distributed from Africa to Indian Ocean islands, from Southwest Asia to Southeast Asia (Silva, Basson & Moe 1996) including China (Tseng, 1984), Japan (Yoshida, Nakajima & Nakata, 1990) and Taiwan (Chiang, 1985), and numerous Pacific islands including the Federated States of Micronesia, Guam (Meneses & Abbott, 1987), Mariana and Northern Mariana Islands (Meneses & Abbott, 1987; Tsuda, 1985), Palau (Ohba *et al.*, 2007) and the Solomon Islands (Womersley & Bailey, 1970). In the Hawaiian Islands, this species was first found in Hilo Bay in

1971 and is considered a non-native although the history of its introduction has not been thoroughly evaluated (Russell, 1992; C. M. Smith pers. comm., 2016; Smith, Hunter & Smith, 2002). This species and others in the genus *Gracilaria* are well studied because of their use in the agar industry (e.g. Nelson *et al.*, 1983; Raikar *et al.*, 2001).

The invasive status of *G. salicornia* in Hawai‘i derives from this plant being particularly resilient, capable of growing on hard substrates, able to acclimate to diurnal and seasonal ranges of temperatures, salinities, and light levels found in the Hawaiian Islands, as well as survive hour-long periods of desiccation (Beach *et al.*, 1997; Smith *et al.*, 2004; Phooprong, Ogawa & Hayashizaki, 2007; Martinez, Smith & Richmond, 2012). Preliminary tests have shown that *G. salicornia* is not highly preferred as a food for native reef fishes (Stimson, Larned & Conklin, 2001; Conklin, 2007; Okano, 2010). The alga can reproduce via fragments (Smith, Hunter & Smith, 2002). Mats can travel to other uncolonized areas by drifting in the water column (Conklin, 2007). These characteristics have allowed *G. salicornia* to establish in many Hawaiian coastal regions, once introduced.

The distribution around Hawai‘i and ability to spread has been documented from several sources. Established populations have been recorded around the islands of O‘ahu, Moloka‘i, and Hawai‘i Island, with more detailed distribution studies in Kāne‘ohe Bay and Waikīkī on O‘ahu, and the south shore of Moloka‘i (Rodgers & Cox, 1999; Smith *et al.*, 2004; Nelson *et al.*, 2009). Rodgers and Cox (1999) reported an ability to spread at the rate of 260-280 m y^{-1}, and Conklin (2007) noted the ability of mats to drift in the current and establish in new areas or recolonize removal sites.

Smith *et al.* (2004) provide a broad overview of the ecology of *G. salicornia* in Waikīkī, Hawai‘i, reporting 5.2 kg m^{-2} wet weight as the average biomass per area and an apical growth

rate of 0.04 g g^{-1} d^{-1} or 10.8% per day in the field. The apical growth rate reported is much higher than found for *in situ* field mats from Molokaʻi (0.03% - 1.28% per day, Nelson *et al.*, 2009), likely associated with field conditions and self-shading. Nelson *et al.* (2009) also corroborate the standing biomass per area, reporting the average biomass on reefs in Molokaʻi as approximately 5.6 kg m^{-2} wet weight. The alga's resilience to extreme salinity and temperature variation was shown by both Smith *et al.* (2004) and Phooprong, Ogawa & Hayashizaki (2007), with the ability to survive a one hour exposure to 0-50 ppt salinity and 8-37 ^{o}C. *G. salicornia* can live in many different light environments, and changes pigment concentrations to photoacclimate, giving thalli diverse colors (Beach *et al.* 1997). Yellow-orange tissue in the canopy of mounds, green tissue in the middle, and dark red-purple tissue in the understory signifies acclimation to high, intermediate, and low irradiance exposure, respectively (Beach *et al.* 1997).

The resilience exhibited by *G. salicornia* has hindered management efforts for control and removal. In a study of the efficacy of control techniques, use of temperature, salinity, desiccation, and chemical management techniques were ultimately dismissed as viable options out of concern for collateral damage to other reef species (Smith *et al.*, 2004). Manual removal is a feasible but labor-intensive control option: 2000 person-hours divided among 400 volunteers to remove 20,000 kg of algae (Smith *et al.*, 2004). Several studies have looked at herbivore grazing as a possible control strategy, with mixed results. Herbivorous fishes were shown to graze on the algae but when given a choice generally prefer other algal species (Smith *et al.*, 2004; Conklin, 2007; Okano, 2010). Marine managers have found that one effective management technique is the rearing and out-planting of a native sea urchin *Tripneustes gratilla* as a grazer for controlling algal growth (Stimson, Cunha & Philippoff, 2007; Westbrook *et al.*, 2015; Neilson *et al.*, in prep). This technique has shown strong success in Kāneʻohe Bay, where marine managers have

focused their efforts on removing the invasive species of *Eucheuma* and *Kappaphycus* through manual removal with aide of a boat-operated vacuum hose, yet have additionally decreased the percent cover of *G. salicornia* by out-planting urchins (Neilson *et al.*, in prep).

Recently, in March 2017, scientists observed several green sea turtles (*Chelonia mydas*) in the back lagoon of Moku o Loʻe island in Kāneʻohe Bay consuming large amounts of *G. salicornia* (Bahr *et al.*, in prep). According to visual benthic surveys, *C. mydas* consumed 41% of *G. salicornia* across 32 sites around the island, with 99% consumption in some areas (Bahr *et al.*, in prep). Although *G. salicornia* has been recorded as a significant part of the *C. mydas* diet in Kāneʻohe Bay (Russell & Balazs, 2009), *C. mydas* had not been observed in this area for 25 years, despite the establishment of *G. salicornia* beds in the back lagoon of Moku O Loʻe circa 1996 – long after the initial introduction of that alga (Rodgers & Cox, 1999; Bahr *et al.*, in prep.). An increase in nesting females during 2013 may have increased recruitment, which may in turn be the reason for the voracious foraging turtles observed here and another means of top-down control of *G. salicornia* (Bahr *et al.*, in prep).

Climate change and macroalgae

Because corals and macroalgae compete for space and possibly other resources, the deterioration of coral condition could result in a large-scale phase shift toward macroalgal dominated ecosystems (Hoegh-Guldberg *et al.*, 2007; Hughes *et al.*, 2007; Koch *et al.*, 2013; Kroeker, Micheli & Gambi, 2013). For example, a decrease in coral cover and recruitment, and an increase in coral mortality were linked to a macroalgal phase shift after an intense bleaching event on the Great Barrier Reef (Hughes *et al.*, 2007). Sites examined close to a naturally occurring CO_2 vent showed increased cover by fleshy macroalgae, compared to coral-dominated control sites (Enochs *et al.*, 2015). Compared to the wealth of studies conducted on corals under

climate change scenarios, there are relatively few studies documenting macroalgal responses to any of the global change parameters (Gao, Helbling & Häder, 2012; Harley *et al.*, 2012; Koch *et al.*, 2013; Ji *et al.*, 2016). Future marine resource managers may be responsible for more algal-dominated ecosystems. Thus, it becomes increasingly important to know how these ecosystems will operate under conditions that reflect the changes that are underway.

At a local level, understanding the potential impacts of climate change on *G. salicornia* and other invasive algae could help us to predict some of the changes that are quickly manifesting in our ecosystem. Hawai‘i’s coral reef ecosystems protect the shorelines, support ecosystem diversity, help feed Hawai‘i’s people, and provide economic benefits by promoting tourism (Moberg & Folke, 1999; Cesar & van Beukering, 2004). Confronted with the loss of this significant resource, it is important to know how a major competitor - an invasive macroalga - will fare under these changing conditions. The following sections summarize the effects of increased temperature and increased pCO_2 on macroalgae, with emphasis on species in the genus *Gracilaria*.

Temperature and macroalgae

Many studies concerning the effects of temperature on algae focus on the immediate reaction of algae to different temperatures rather than on long term effects. For example, many studies explore Q_{10} values, or the rate of a process (such as photosynthesis, respiration, or growth) at T_1 °C divided by the rate of the same process at T_1 - 10°C (Davison, 1991). These studies generally grow the algae at an ambient temperature, and then expose the algae to various temperature regimes for a short period of time to see how the algae react to sudden and extreme changes. Q_{10} studies across terrestrial and marine botany generate physiological performance curves, with lowered performance at temperature extremes and a bell-shaped curve with maxima

at the species-specific optimal temperature (Laing, 2012; Harley *et al.*, 2012). Though Q_{10} studies are clearly informative, they should be approached with caution when estimating the effects of gradual global warming of sea surface temperatures. Short-term (minutes to hours) response of algae to temperature change is often contradictory to long-term (days to weeks) response (Li, 1980; Davison, 1991). Thus, experiments looking to mimic the effects of climate change should allow time for the organism to acclimate to treatment conditions.

Determining the long-term (days to weeks) effects of temperature change is more challenging as it consumes more time and resources. However, studies that allow an acclimation period are more representative of how organisms will react to such changes over a longer period (Koch *et al.*, 2013). Unfortunately, there is very little information on the length of time needed for algae to acclimate to temperature treatment conditions (Davison, 1991). Many algal studies looking at long-term effects of temperature will acclimate samples to treatments for several weeks, but there is evidence that acclimation begins within hours of exposure to a temperature change (Davison, 1991). Multi-week acclimation periods in manipulative experiments can be problematic in climate change research for experimental design reasons. Climate change researchers often apply treatments using large header chambers that flow into individual tanks. To avoid pseudo replication, these experiments should be replicated several times to determine if any observed effects are a result of treatment conditions or the processes taking place in a mixing chamber (Wernberg, Smale & Thomsen, 2012). Including a multi-week acclimation period into each experimental trial limits the number of trials that can be performed in a specific amount of time, and therefore experimental runs need to be planned accordingly.

A few patterns emerge from long-term studies that have either included a temperature acclimation period or grown the plant samples at the treatment temperature. When comparing

algal samples of the same species grown at a range of different temperatures, those grown at lower temperatures had higher maximum photosynthetic rates (Davison, 1991). Regardless of the temperature at which the algae were grown, the optimal temperature for photosynthesis was usually higher than the optimal temperature for growth, which highlights the fact that one process cannot be used as a direct proxy for the other in temperature experiments (Li, 1980; Lapointe, Tenore & Dawes, 1984; Davison, 1991; Koch *et al.*, 2013). Li (1980) describes growth in phytoplankton as "the integrated expression of cellular processes," meaning growth rates depend not only on photosynthetic rates, but also on other factors such as respiration and decay. Therefore, discrepancies in optimal temperature values for growth and photosynthesis may reflect an increased cost in respiration and excretion at higher temperatures (Li, 1980; Lapointe, Tenore & Dawes, 1984; Robarts & Zohary, 1987). In manipulative experiments with phytoplankton grown for several generations under different temperatures, the temperature for maximum photosynthetic rate was usually several degrees higher than the temperature at which phytoplankton were grown (Li, 1980). The relationship between respiration and temperature is not well studied in macroalgae, although research indicates that increases in temperature correlate with increases in dark respiration and photorespiration (Lapointe, Tenore & Dawes, 1984; Koch *et al.*, 2013). Several studies on cyanobacteria suggest an exponential relationship between water temperature and respiration rates (reviewed in Robarts & Zohary, 1987).

Carbon dioxide and macroalgae

Inorganic carbon in aquatic habitats exists in three principle forms: carbon dioxide (CO_2), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}). These carbon compounds exist in equilibrium with each other according to Equations 1.1 and 1.2 (Dickson, Sabine, & Christian, 2007). In seawater at normal pH (8.0-8.2), the concentration of CO_2 is approximately 10 μM, which is low

compared to the 2000 μM concentration of HCO_3^-. Adding CO_2 to seawater acidifies the water by increasing the concentration of dissolved hydrogen ions (H^+), lowering the pH. Equation 1.1 indicates that seawater with higher pH values will have a higher ratio of [HCO_3^-] to [CO_2], whereas seawater with lower pH values will have a lower ratio, favoring CO_2. Understanding the ways these carbon compounds interact is necessary for interpreting how chemistry changes can affect life processes.

Equation 1.1: $CO_{2(aq)} + H_2O \leftrightarrow HCO_3^-{}_{(aq)} + H^+{}_{(aq)}$

Equation 1.2: $HCO_3^-{}_{(aq)} \leftrightarrow CO_3^{2-}{}_{(aq)} + H^+{}_{(aq)}$

Carbon dioxide availability is integral to all plant photosynthesis. In the balanced equation of photosynthesis, carbon dioxide combines with water and solar energy within plant cells to produce glucose. For some algae, the amount of dissolved inorganic carbon (DIC) available in seawater is not enough to maximize growth; these plants are termed "carbon limited" (Koch *et al.*, 2013). Although CO_2 is the carbon compounds necessary for photosynthesis, some algae have evolved ways to use the more abundant compound, HCO_3^- (Koch *et al.*, 2013; Raven & Beardall, 2014). For example, many macroalgae use the enzyme carbonic anhydrase (CA) to convert HCO_3^- into CO_2, which then passively diffuses into algal cells for photosynthetic use (Koch *et al.*, 2013; Raven & Beardall, 2014). Changes in the concentrations of seawater carbon molecules may have widespread, species-specific effects depending on how different algae take in carbon for photosynthesis and whether they are carbon limited at current DIC levels.

There is a wealth of research on how increased carbon concentrations affect macroalgae but it is questionable how much of this research is applicable to global climate change scenarios.

Most of the research on this subject conducted before the year 2000 focused on inorganic carbon as a nutrient. Manipulative experiments on macroalgae used treatments of enhanced CO_2 or HCO_3^- to determine the effects of increased DIC as a fertilizer for algal growth, but most of these treatments exponentially exceeded the predicted pCO_2 under climate change scenarios (e.g. DeBusk & Ryther, 1984; García-Sanchez, Fernández & Niell, 1994). Other experiments analyzed the short-term effects (minutes to hours) of exposure to high pCO_2 conditions (e.g. Haglund *et al.*, 1992; Rivers & Peckol, 1995). Because of their short time scales and extreme carbon concentrations, both of these methodologies should be reviewed with caution when attempting to assess how long-term and gradual increases of pCO_2 associated with global climate change will affect macroalgae.

Several studies have examined the effect of pH and inorganic carbon concentration on members of red algal genus *Gracilaria*. For at least three species (*G. tikvahiae*, *G. secundata,* and *G. lemaneiformis*), increasing the amount of available carbon, through CO_2 bubbling or $NaHCO_3$ supplements, and maintaining a pH between 7.85-8.00 led to increases in growth rates (DeBusk & Ryther, 1984; Lignell & Pedersén, 1989; Xu, Zou & Gao, 2010). Growth rates sharply declined at pH values above 8.6 and gradually declined at pH values below 7.8, regardless of carbon abundance (DeBusk & Ryther, 1984; Lignell & Pedersén, 1989). Multiple species of *Gracilaria*, including *G. lemaneiformis*, *G. secundata*, *G. tenuistipitata*, and *G. tikvahiae*, have shown the capacity to use HCO_3^- as a carbon source for photosynthesis through the use of CA. These species however do show a higher affinity for CO_2 (Lignell & Pedersén, 1989; Haglund *et al.*, 1992; García-Sanchéz, Fernández, & Niell, 1994; Xu, Zou & Gao, 2010). One study found lowered growth rates, photosynthetic rates, pigment content, and Rubisco content in *G. tenuistipitata* grown at high DIC concentrations; however, the treatments used were

50x the highest predicted levels of CO_2 under global climate change scenarios (García-Sanchéz, Fernández, & Niell, 1994).

Research oriented specifically toward climate change scenarios with macroalgae have shown a positive, neutral, or negative relationship between pCO_2 and growth/photosynthesis for an alga, based on species differences (Table 1.1; Gao, Helbling & Häder, 2012; Harley *et al.*, 2012; Koch *et al.*, 2013; Johnson, Price & Smith, 2014; Ji *et al.* 2016). No studies have been conducted on *G. salicornia* specifically, and few studies have looked at the interaction between both pCO_2 and temperature on the photosynthesis and respiration of a fleshy macroalgae.

Proposal

This research will examine the effects of increased temperature and increased pCO_2 on the respiration and photosynthetic rates of *G. salicornia*. The amount of increase in temperature and pCO_2 will be set by climate change projection models for the year 2100. Response variables will be analyzed for interactive effects between temperature and pCO_2. The experiment will be carried out in outdoor, flow-through aquaria to best replicate natural light conditions, day/night period, salinity, ambient temperature, ambient pH and water chemistry, and environmental variation.

Table 1.1 Literature summary: effect of global climate change CO_2 levels on fleshy rhodophytes

Reference	Species	Irradiance source	Experiment type	Nutrients added	Variables measured	Effects of elevated CO_2
Israel & Hophy (2002)	*Hypnea cornuta*	Natural sunlight	Natural field setting	No	Growth	negative
	Hypnea musciformis	Unspecified	Tanks with aeration	Yes	Growth	neutral
	Porphyra sp.	Unspecified	Tanks with aeration	Yes	Growth	neutral
	Pterocladia capillacea	Unspecified	Tanks with aeration	Yes	Growth	neutral
	Pterocladia capillacea	Natural sunlight	Natural field setting	No	Growth	neutral
	Solieria sp.	Unspecified	Tanks with aeration	Yes	Growth	neutral
Johnson, Price & Smith (2014)	*Acanthophora spicifera*	Natural sunlight with shade cloth: to simulate 5 m ocean depth	Tanks with aeration	No	Growth	neutral
					Photosyn-thesis	negative
	Hypnea pannosa	Natural sunlight with shade cloth: to simulate 5 m ocean depth	Tanks with aeration	No	Growth	positive
					Photosyn-thesis	neutral
Kram *et al.*, (2016)	*Plocamium cartilagineum*	Full-spectrum fluorescent bulbs	Flow-through tanks with aeration	No	Growth	positive
Ho & Carpenter (2017)	*Amansia rhodantha*	Natural sunlight with fiberglass covers: attenuation to simulate back reef (<3 m depth)	Flow-through flumes	No	Growth	neutral

Literature Cited

Ampou EE, Johan O, Menkes CE, Niño F, Birol F, Ouillon S, Andréfouët S. 2017. Coral mortality induced by the 2015-2016 El-Niño in Indonesia: the effect of rapid sea level fall. *Biogeosciences* 14:817-826.

Anlauf H, D'Croz L, O'Dea A. 2011. A corrosive concoction: the combined effects of ocean warming and acidification on the early growth of a stony coral are multiplicative. *Journal of Experimental Marine Biology and Ecology* 397:13–20. DOI: 10.1016/j.jembe.2010.11.009.

Bahr KD, Coffey DM, Rodgers K, Balazs GH. In prep. Green sea turtles on the move for food: *Honu* in Hawai‘i grazing their way into new habitats.

Bahr KD, Jokiel PL, Rodgers KS. 2015. The 2014 coral bleaching and freshwater flood events in Kāne‘ohe Bay, Hawai‘i. *PeerJ* 3:e1136. DOI: 10.7717/peerj.1136.

Bahr KD, Jokiel PL, Rodgers KS. 2016. Relative sensitivity of five Hawaiian coral species to high temperature under high-pCO_2 conditions. *Coral Reefs* 35:729–738. DOI: 10.1007/s00338-016-1405-4.

Beach KS, Borgeas HB, Nishimura NJ, Smith CM. 1997. In vivo absorbance spectra and the ecophysiology of reef macroalgae. *Coral Reefs* 16:21–28. DOI: 10.1007/s003380050055.

Cesar H, van Beukering P, Pintz S, Dierking J. 2002. Economic valuation of the coral reefs of Hawai‘i: final report. Report to: University of Hawai‘i for the Hawai‘i Coral Reef Initiative Research Program. *Available at* https://www.coris.noaa.gov/portals/pdfs/hicesar.pdf (Accessed 20 June 2017). pp1-123.

Cesar HSJ, van Beukering PJH. 2004. Economic valuation of the coral reefs of Hawai‘i. *Pacific Science* 58:231-242. DOI: 10.1353/psc.2004.0014.

Chiang YM. 1985. *Gracilaria* from Taiwan: key, list and distribution of the species. In: Abbott IA, Norris JN, eds. *Taxonomy of economic seaweeds with reference to some Pacific and Caribbean species*. 1:81-83. California Sea Grant College Program. no.T-CSGCP-011.

Conklin EJ. 2007. The influence of preferential foraging, alien algal species, and predation risk on the interaction between herbivorous fishes and reef macroalgae. *Ph D. Dissertation, University of Hawai‘i at Mānoa.* pp. 1-241.

Dailer ML, Smith JE, Smith CM. 2012. Responses of bloom forming and non-bloom forming macroalgae to nutrient enrichment in Hawai‘i, USA. *Harmful Algae* 17:111-125.

Davidson AD, Campbell ML, Hewitt CL, Schaffelke B. 2015. Assessing the impacts of nonindigenous marine macroalgae: an update of current knowledge. *Botanica Marina* 58:55-79.

Davison IR 1991. Environmental effects on algal photosynthesis: temperature. *Journal of Phycology* 27:2–8. DOI: 10.1111/j.0022-3646.1991.00002.x.

DeBusk T, Ryther J. 1984. Effects of seawater exchange, pH and carbon supply on the growth of *Gracilaria tikvahiae* (Rhodophyceae) in large-scale cultures. *Botanica Marina* 27:357-362.

DeCarlo TM, Cohen AL, Wong GT, Davis KA, Lohmann P, Soong K. 2017. Mass coral mortality under local amplification of 2° C ocean warming. *Scientific Reports* 7:44586.

Dickson AG, Sabine CL, and Christian JR, eds. 2007. Guide to best practices for ocean CO_2 measurements. *PICES Special Publication* 3:1-191.

Enochs IC, Manzello DP, Donham EM, Kolodziej G, Okano R, Johnston L, Young C, Iguel J, Edwards CB, Fox MD, Valentino L, Johnson S, Benavente D, Clark SJ, Carlton R, Burton T, Eynaud Y, Price NN. 2015. Shift from coral to macroalgae dominance on a volcanically acidified reef. *Nature Climate Change* 5:1083-1089. DOI: 10.1038/nclimate2758

Fukunaga A, Peyton KA, Thomas FIM. 2014. Epifaunal community structure and ammonium uptake compared for the invasive algae, *Gracilaria salicornia* and *Acanthophora specifera*, and the native alga, *Padina thivyi*. *Journal of Experimental Marine Biology and Ecology* 456:78-86.

Gao K, Helbling EW, Häder DP. 2012. Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming. *Marine Ecology Progress Series* 470:167-189. DOI: 0.3354/meps10043

García-Sánchez M, Fernández J, Niell X. 1994. Effect of inorganic carbon supply on the photosynthetic physiology of *Gracilaria tenuistipitata*. *Planta* 194:55-61.

Godwin S, Rodgers K, Jokiel PL. 2006. Reducing potential impact of invasive marine species in the Northwestern Hawaiian Islands marine national monument. Report to: Northwest Hawaiian Islands Marine National Monument Administration. no. DOI, NOAA, National Ocean Service MOA 2005-008/6882Amendment No. 001. *Available at* http://www.academia.edu/13857287/Reducing_potential_impact_of_invasive_marine_species_in_the_northwestern_hawaiian_islands_marine_national_monument (Accessed 6 June 2017). pp1-66.

Haglund K, Björk M, Ramazanov Z, García-Reina G, Pedersén M. 1992. Role of carbonic anhydrase in photosynthesis and inorganic-carbon assimilation in the red alga *Gracilaria tenuistipitata*. *Planta* 187:1–8. DOI: 10.1007/BF00201951.

Harley CDG, Anderson KM, Demes KW, Jorve JP, Kordas RL, Coyle TA, Graham MH. 2012. Effects of climate change on global seaweed communities. *Journal of Phycology*

48:1064–1078. DOI: 10.1111/j.1529-8817.2012.01224.x.

Ho M, Carpenter RC. 2017. Differential growth responses to water flow and reduced pH in tropical marine macroalgae. *Journal of Experimental Marine Biology and Ecology* 491:58–65. DOI: 10.1016/j.jembe.2017.03.009.

Hoegh-Guldberg O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research* 50:839–866. DOI: 10.1071/mf99078.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME. 2007. Coral reefs under rapid climate change and ocean acidification. *Science* 318:1737–1742. DOI: 10.1126/science.1152509.

Hoegh-Guldberg O, Polockzanska ES, Skirving W, Dove S. 2017. Coral reef ecosystems under climate change and ocean acidification. *Frontiers in Marine Science* 4:158. DOI: 10.3389/fmars.2017.00158

Huang DW, Roy K. 2015. The future of evolutionary diversity in reef corals. *Philosophical Transactions of the Royal Society B* 370: 20140010. DOI: 10.1098/rstb.2014.0010

Hughes TP, Kerry JT, Álvarez-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH, Babcock RC, Beger M, Bellwood DR, Berkelmans R, Bridge TC. 2017. Global warming and recurrent mass bleaching of corals. *Nature* 543:373-7. DOI: 10.1038/nature21707.

Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskyj N, Pratchett MS, Steneck RS, Willis B. 2007. Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Current Biology* 17:360–365. DOI: 10.1016/j.cub.2006.12.049.

Hunter CL, Evans CW. 1995. Coral reefs in Kaneohe Bay, Hawaii: two centuries of western influence and two decades of data. *Bulletin of Marine Science* 57: 501-515.

Intergovernmental Panel on Climate Change (IPCC). 2014. Pachauri RK, Meyer LA, eds. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC, Geneva, Switzerland, 1-151.

Israel A, Hophy M. 2002. Growth, photosynthetic properties and Rubisco activities and amounts of marine macroalgae grown under current and elevated seawater CO_2 concentrations. *Global Change Biology* 8:831–840. DOI: 10.1046/j.1365-2486.2002.00518.x.

Ji Y, Xu Z, Zou D, Gao K. 2016. Ecophysiological responses of marine macroalgae to climate change factors. *Journal of Applied Phycology* 28:2953–2967. DOI: 10.1007/s10811-016-0840-5.

Johnson MD, Price NN, Smith JE. 2014. Contrasting effects of ocean acidification on tropical fleshy and calcareous algae. *PeerJ* 2:e411. DOI: 10.7717/peerj.411.

Jokiel PL, Brown EK. 2004. Global warming, regional trends and inshore environmental conditions influence coral bleaching in Hawai‘i. *Global Change Biology* 10:1627–1641. DOI: 10.1111/j.1365-2486.2004.00836.x.

Koch M, Bowes G, Ross C, Zhang XH. 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology* 19:103–132. DOI: 10.1111/j.1365-2486.2012.02791.x.

Kram SL, Price NN, Donham EM, Johnson MD, Kelly ELA, Hamilton SL, Smith JE. 2016. Variable responses of temperate calcified and fleshy macroalgae to elevated pCO_2 and warming. *ICES Journal of Marine Science* 73:693–703. DOI: 10.1093/icesjms/fsv168.

Kroeker KJ, Gambi MC, Micheli F. 2013. Community dynamics and ecosystem simplification in a high-CO_2 ocean. *Proceedings of the National Academy of Science* 110:12721-12726.

Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso JP. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology* 19:1884-1896. DOI: 10.1111/gcb.12179.

Kroeker KJ, Micheli F, Gambi MC. 2013. Ocean acidification causes ecosystem shifts via altered competitive interactions. *Nature Climate Change* 3:156–159. DOI: 10.1038/nclimate1680.

Laing WA. 2012. Temperature and light response curves for photosynthesis in kiwifruit (*Actinidia chinensis*) cv. Hayward. *New Zealand Journal of Agricultural Research* 28(1):117-124. DOI: 10.1080/00288233.1985.10427004.

Lapointe BE, Tenore KR, Dawes CJ. 1984. Interactions between light and temperature on the physiological ecology of *Gracilaria tikvahiae* (Gigartinales: Rhodophyta). *Marine Biology* 80:161–170. DOI: 10.1007/BF02180183.

Larned ST.1998. Nitrogen- versus phosphorus-limited growthand sources of nutrients for coral reef macroalgae. *Marine Biology* 132:409–421.

Li W. 1980. Temperature adaptation in phytoplankton: cellular and photosynthetic characteristics. In: Falkowski P, ed. *Primary Productivity in the Sea*. Springer US, pp 259-277.

Lignell Å, Pedersén M. 1989. Effects of pH and inorganic carbon concentration on growth of *Gracilaria secundata*. *British Phycological Journal* 24:83-89. DOI: 10.1080/00071618900650071.

Martinez JA. 2012. The physical and physiological impacts of the invasive red macroalga *Gracilaria salicornia* Dawson to coral reef habitat quality and coral health. *Ph. D. Dissertation, University of Hawai'i at Mānoa*, pp 1-127.

Martinez JA, Smith CM, Richmond RH. 2012. Invasive algal mats degrade coral reef physical habitat quality. *Estuarine, Coastal and Shelf Science* 99:42–49. DOI: 10.1016/j.ecss.2011.12.022.

Meneses I, Abbott IA. 1987. *Gracilaria* and *Polycavernosa* (Rhodophyta) from Micronesia. *Micronesica* 20:187-200.

Moberg F, Folke C. 1999. Ecological goods and services of coral reef ecosystems. *Ecological economics* 29:215–233. DOI: 10.1016/S0921-8009(99)00009-9.

Munday PL, Jones GP, Pratchett MS, Williams AJ. 2008. Climate change and the future for coral reef fishes. *Fish and Fisheries* 9:261-85.

Neilson BJ, Wall CB, Mancini FT, Gewecke CA. In prep. Herbivore biocontrol and manual removal successfully reduce invasive macroalgae on coral reefs.

Nelson SG, Glenn EP, Moore D, Ambrose B. 2009. Growth and distribution of the macroalgae *Gracilaria salicornia* and *G. parvispora* (Rhodophyta) established from aquaculture introductions at Moloka'i, Hawai'i. *Pacific Science* 63:383–396. DOI: 10.2984/049.063.0307.

Nelson S, Yang S, Chiang Y. 1983. Yield and quality of agar from species of *Gracilaria* (Rhodophyta) collected from Taiwan and Micronesia. *Botanica Marina* 26:361-366.

Ohba H, Victor S, Golbuu Y, Yukihara H. 2007. *Tropical marine plants of Palau*. Palau International Coral Reef Center; Japan International Cooperation Agency. pp. 1-153.

Okano R. 2010. Potential influences of submarine groundwater discharge, nutrients, and herbivory on Hawaiian reef algae. *Ph D Dissertation, University of Hawai'i at Mānoa*, pp. 1-164.

Okazaki RR, Towle EK, Hooidonk R, Mor C, Winter RN, Piggot AM, Cunning R, Baker AC, Klaus JS, Swart PK, Langdon C. 2017. Species-specific responses to climate change and community composition determine future calcification rates of Florida Keys reefs. *Global Change Biology* 23:1023–1035. DOI: 10.1111/gcb.13481.

Phooprong S, Ogawa H, Hayashizaki K. 2007. Photosynthetic and respiratory responses of *Gracilaria salicornia* (C. Ag.) Dawson (Gracilariales, Rhodophyta) from Thailand and Japan. *Journal of Applied Phycology* 19:795–801. DOI: 10.1007/s10811-007-9194-3.

Pörtner, HO, Karl DM, Boyd PW, Cheung WWL, Lluch-Cota SE, Nojiri Y, Schmidt DN, Zavialov PO. 2014. Ocean systems. In: Field, CB, Barros VR, Dokken DJ, Mach KJ,

Mastrandrea MD, Bilir TE, Chatterjee M, Ebi KL, Estrada YO, Genova RC, Girma B, Kissel ES, Levy AN, MacCracken S, Mastrandrea PR, White LL, eds. *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. pp. 411-484.

Porzio L, Buia MC, Hall-Spencer JM. 2011. Effects of ocean acidification on macroalgal communities. *Journal of Experimental Marine Biology and Ecology* 400:278–287. DOI: 10.1594/PANGAEA.728715.

Przeslawski R, Byrne M, Mellin C. 2015. A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Global Change Biology* 21:2122–2140. DOI: 10.1111/gcb.12833.

Raikar S, Iima M, Fujita Y. 2001. Effect of temperature, salinity and light intensity on the growth of *Gracilaria* spp. (Gracilariales, Rhodophyta) from Japan, Malaysia and India. *Indian Journal of Geo-Marine Sciences* 30:98-104.

Raven JA, Beardall J. 2014. CO_2 concentrating mechanisms and environmental change. *Aquatic Botany* 118:24-37.

Rivers J, Peckol P. 1995. Interactive effects of nitrogen and dissolved inorganic carbon on photosynthesis, growth, and ammonium uptake of the macroalgae *Cladophora vagabunda* and *Gracilaria tikvahiae*. *Marine Biology* 121:747-753.

Robarts RD, Zohary T. 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *New Zealand Journal of Marine and Freshwater Research* 21:391–399. DOI: 10.1080/00288330.1987.9516235.

Rodgers SK, Bahr KD, Jokiel PJ, Richards Donà A. 2017. Patterns of bleaching and mortality following widespread warming events in 2014 and 2015 at the Hanauma Bay Nature Preserve, Hawai‘i. *PeerJ* 5:e3355. DOI 10.7717/peerj.3355.

Rodgers SK, Cox EF. 1999. Rate of spread of introduced rhodophytes *Kappaphycus alvarezii, Kappaphycus striatum*, and *Gracilaria salicornia* and their current distributions in Kāne‘ohe bay, O‘ahu, Hawai‘i. *Pacific Science* 53:232–241.

Russell, DJ. 1992. The ecological invasion of Hawaiian reefs by two marine red algae, *Acanthophora spicifera* (Vahl) Boerg. and *Hypnea musciformis* (Wulfen) J. Ag., and their association with two native species, *Laurencia nidifica* J. Ag. and *Hypnea cervicornis* J. Ag. *ICES Marine Science Symposium* 194:110-125.

Russell DJ, Balazs GH. 2009. Dietary shifts by green turtles (Chelonia mydas) in the Kāne‘ohe Bay region of the Hawaiian Islands: A 28-Year Study1. *Pacific Science* 63:181–192. DOI: 10.2984/049.063.0202.

Silva PC, Basson PW, Moe RL. 1996. *Catalogue of the benthic marine algae of the Indian Ocean*. Berkeley: University of California Press. pp 1-1259.

Smith JE, Hunter CL, Conklin EJ, Most R, Sauvage T, Squair C, Smith CM. 2004. Ecology of the invasive red alga *Gracilaria salicornia* (Rhodophyta) on O'ahu, Hawai'i. *Pacific Science* 58:325–343.

Smith JE, Hunter CL, Smith CM. 2002. Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian Islands. *Pacific Science* 56:299–315. DOI: 10.1017/CBO9780511751790.021.

Smith SV, Kimmerer WJ, Laws EA, Brock RE, Walsh TW. 1981. Kaneohe Bay sewage diversion experiment: perspectives on ecosystem responses to nutritional perturbation. *Pacific Science* 35:279-395.

Stimson J, Cunha T, Philippoff J. 2007. Food preferences and related behavior of the browsing sea urchin *Tripneustes gratilla* (Linnaeus) and its potential for use as a biological control agent. *Marine Biology* 151:1761–1772. DOI: 10.1007/s00227-007-0628-x.

Stimson J, Larned S, Conklin E. 2001. Effects of herbivory, nutrient levels, and introduced algae on the distribution and abundance of the invasive macroalga *Dictyosphaeria cavernosa* in Kāne'ohe Bay, Hawai'i. *Coral Reefs* 19:343–357. DOI: 10.1007/s003380000123.

Stimson J, Larned S, McDermid K. 1996. Seasonal growth of the coral reef macroalga *Dictyosphaeria cavernosa* (Forskål) Børgesen and the effects of nutrient availability, temperature and herbivory on growth rate. *Journal of Experimental Marine Biology and Ecology* 196:53-57.

Tseng C. 1984. *Common seaweeds of China*. Beijing, China: Science Press. pp 1-318.

Tsuda, RT. 1985. *Gracilaria* from Micronesia: key, list and distribution of the species. In: Abbott IA, Norris JN, eds. In: *Taxonomy of economic seaweeds with reference to some Pacific and Caribbean species*. 1:81-83. California Sea Grant College Program. no. T-CSGCP-011.

Wernberg T, Smale DA, Thomsen MS. 2012. A decade of climate change experiments on marine organisms: procedures, patterns and problems. *Global Change Biology* 18:1491–1498. DOI: 10.1111/j.1365-2486.2012.02656.x.

Westbrook CE, Ringang RR, Cantero SMA, HDAR & TNC Urchin Team, Toonen RJ. 2015. Survivorship and feeding preferences among size classes of outplanted sea urchins, *Tripneustes gratilla*, and possible use as biocontrol for invasive alien algae. *PeerJ* 3:e1235. DOI:10.7717/peerj.1235.

Wilkinson C, Lindén O, Cesar H, Hodgson G, Rubens J, Strong AE. 1999. Ecological and socioeconomic impacts of 1998 coral mortality in the Indian Ocean: An ENSO impact and a warning of future change? *Ambio* 28:188-96.

Womersley H, Bailey A. 1970. Marine algae of the Solomon Islands. *Philosophical Transactions of the Royal Society B: Biological Sciences* 259:257-352.

Xu Z, Zou D, Gao K. 2010. Effects of elevated CO_2 and phosphorus supply on growth, photosynthesis and nutrient uptake in the marine macroalga *Gracilaria lemaneiformis* (Rhodophyta). *Botanica Marina* 53:123-129.

Yoshida T, Nakajima YT, Nakata Y. 1990. Check-list of marine algae of Japan (revised in 1990). *Japanese Journal of Phycology* 38:269-320.

Chapter 2: The effects of increased temperature and pCO_2 on respiration and photosynthetic rates of *Gracilaria salicornia*

Introduction

With the warming and acidification of the oceans, tropical coral reef ecosystems are expected to change dramatically over the next decades (Hoegh-Guldberg *et al.*, 2007; Pörtner *et al.*, 2014). Warmer sea surface temperatures are directly linked to large-scale coral bleaching events and increased pCO_2 in the ocean is disrupting the ability to build strong calcified skeletons (Jokiel & Brown, 2004; Hoegh-Guldberg, 1999; Hoegh-Guldberg *et al.*, 2007; Doney *et al.*, 2009; Bahr, Jokiel & Rodgers, 2015; Rodgers *et al.*, 2017). Within the past three years, a worldwide series of bleaching events have caused damage to corals in many areas including Australia (Hughes *et al.*, 2017), Asia (Ampou *et al.*, 2017; DeCarlo *et al.*, 2017), and in the Hawaiian Islands (Bahr, Jokiel & Rodgers, 2015; Rodgers *et al.*, 2017). Beyond corals, other calcifying organisms, such as mollusks, echinoderms, crustaceans, coralline algae, and coccolithophores, will be affected by the chemical changes inherent in ocean acidification to varying degrees (Kroeker *et al.*, 2013; Pörtner *et al.*, 2014). These major environmental changes are predicted to devastate much of the world's remaining coral reefs (Hoegh-Guldberg *et al.*, 2007).

Corals and fleshy macroalgae live in a balance dictated in part by competition. They compete for shared resources including space and sunlight (McCook, Jompa & Diaz-Pulido, 2001). In the aftermath of a bleaching event, Australian researchers found that macroalgae overgrew corals and inhibited coral recovery when herbivorous fish were excluded, demonstrating the potential for macroalgal phase shifts in overfished areas after a disturbance event (Hughes *et al.*, 2007). Surveys of sites along a naturally occurring CO_2 gradient near a

volcanic vent demonstrated a shift from high coral cover in ambient seawater to algal dominance (macroalgae and turf algae) in more acidified zones (Enochs *et al.*, 2015). If the next century brings major competitive disadvantages for corals, macroalgae may outcompete corals and become dominant on many reefs (Hughes *et al.*, 2007; Hoegh-Guldberg *et al.*, 2007; Porzio, Buia & Hall-Spencer, 2011; Koch *et al.*, 2013; Enochs *et al.*, 2015).

Compared to corals, there is relatively little known about how fleshy macroalgae will respond to climate change (Wernberg, Smale & Thomsen, 2012). Of marine climate change research conducted between years 2000-2009, only 11% focused on macroalgae, compared to over 70% that focused on benthic invertebrates including coral (Wernberg, Smale & Thomsen, 2012). Within the category of macroalgae, focus has been given to calcifying algae including coralline algae, and results have generally shown reduced growth and reduced abundance in high pCO_2 environments (Koch *et al.*, 2013; Kroeker *et al.*, 2013; Kroeker, Micheli & Gambi, 2013; Johnson, Price & Smith, 2014; Kram *et al.*, 2016) There are fewer studies and less clear results regarding climate change and fleshy macroalgae. From short-term manipulative pCO_2 experiments, researchers have found that a small percentage of fleshy macroalgae show an increase in growth rates under high pCO_2 conditions, a few show decreased growth rates, but the majority show no significant response (e.g. Israel & Hophy, 2002; Diaz-Pulido *et al.*, 2011; Johnson, Price & Smith, 2014; Kram *et al.*, 2016; Ho & Carpenter, 2017). The long-term effect of temperature on algal growth is defined by individual optimal growth temperatures and temperature threshold limits, although how these will change with acclimation and adaptation is not well studied (Harley *et al.*, 2012). Our lack of knowledge makes it difficult to predict how macroalgal communities will react to climate change, which is concerning because macroalgae may become the dominant on reefs.

In the Hawaiian Islands, overgrowth by invasive macroalgae has been a problem since the late 1970s, if not earlier (Russell, 1992). At least 19 species of foreign marine macroalgae have been transported purposefully or accidentally to the Hawaiian Islands since the 1950s and some of these algae have shown the capacity for rapid growth and spread (Russell, 1992; Smith, Hunter & Smith, 2002). There are financial, ecological, and social consequences of invasive algal blooms including loss of property value, clean-up and management costs (Cesar *et al.* 2002), competition for native algal niches (Russell, 1992), impacts to native gathering practices, modification of the benthic environment, increased stress to corals (Martinez, Smith & Richmond, 2012), and changes in invertebrate abundances (Fukunaga, Peyton & Thomas, 2014).

One of the most invasive species in Hawai‘i is the rhodophyte *Gracilaria salicornia* (C. Ag.) Dawson (Smith, Hunter & Smith, 2002; Godwin, Rodgers & Jokiel, 2006). The success of *G. salicornia* in Hawai‘i stems in part from the alga’s resilience; it is capable of growing on hard substrates, able to acclimate to diurnal and seasonal ranges of temperatures, salinities, and light levels found in the Hawaiian Islands, as well as survive hour-long periods of desiccation (Beach *et al.*, 1997; Smith *et al.*, 2004; Phooprong, Ogawa & Hayashizaki, 2007; Martinez, Smith & Richmond, 2012). *G. salicornia* is able to spread quickly at the rate of 260-280 m y^{-1} and has established populations around the islands of O‘ahu, Moloka‘i, and Hawai‘i Island, with more detailed distribution studies in Kāne‘ohe Bay and Waikīkī on O‘ahu, and the south shore of Moloka‘i (Rodgers & Cox, 1999; Smith *et al.*, 2004; Nelson *et al.*, 2009). Once established, it grows quickly at the rate of 10.8% per day, covering the substrate in large mounds amounting to over 5.2 kg m^{-2} wet weight in densely covered areas (Smith *et al.* 2004). To this date, no studies have been conducted to see how *G. salicornia* will fare under climate change conditions.

Within the limited research on macroalgae, there is a need for experiments that (1) mimic daily cycles and the large environmental variance found in the tropical coral reef ecosystem, and (2) examine pCO_2 and temperature for interactive effects (Gao, Helbling & Häder, 2012; Koch *et al.*, 2013). This experiment addresses these knowledge gaps by examining the individual and interactive effects of temperature and pCO_2 on algal metabolism in *G. salicornia*. The response variables measured were dark respiration rate via oxygen evolution methods, and the rate of photosynthesis via fluorometric analysis. Outdoor, continuous-flow seawater systems were used in this experiment to capture the environmental variability inherent in natural ecosystems (Jokiel, Bahr & Rodgers, 2014).

Methods

Experimental design

Tissue samples of *Gracilaria salicornia* (C. Agardh) E. Y. Dawson (~4 g wet weight) were collected from a ~60 m^2 collection location near the Coral Reef Ecology Lab on Moku o Loʻe, Oʻahu. To encompass genetic variability, all samples were collected at least 1 m apart. All samples were collected between 0.5-1.5 m depth from healthy, ungrazed tissue of uniform green-gray color. Tissues were hand-cleaned of all macroscopic sediment, epiphytes, and epifauna using soft brushes and metal fine point picks.

Tissues were transported to Ānuenue Fisheries Research Center (AFRC) in seawater-filled, sealed plastic bags within a cooler with ice packs. They were placed in aquaria filled with 5 μm filtered seawater with manipulated pH and temperature, detailed below. On the day of collection, maximum electron transfer rates (ETR_{max}) were measured for each collected tissue using a Pulse Amplitude Moderated Fluorometer (Junior PAM, Walz, Germany). The eight most-similar ETR_{max} readings were used to choose the tissue samples experimental trial, to

lessen the variance from sun/shade acclimation states. Excess tissue samples were discarded. A total of thirty-two tissue samples were used during the four experimental trials.

In the aquarium system, there were two levels of pH: ambient and acidified (~ -0.2 pH units from normal seawater) and two levels of temperature: ambient and heated (~ +1° C from normal seawater) in a full factorial design (Figures 2.1 and 2.2). The increased temperature and acidification simulate potential climate conditions in the year 2100, following the International Panel on Climate Change 2014 report (Pörtner *et al.*, 2014). pH was adjusted by bubbling CO_2 gas into header tanks and a temperature difference was maintained using titanium water heaters submerged in the header tanks, following Jokiel, Bahr & Rodgers (2014), to maintain consistency in all aquaria. Tissues were given approximately 52 h to acclimate to treatment conditions prior to initial measurements of response variables (Lignell & Pedersén, 1989).

Each experimental trial ran for four days from initial collection and exposure to treatment conditions to the final measurements. Subsequently, all tissues were dried until stable dry-weight was achieved (five days). Four experimental trials were conducted between October and December of 2016 (Table 2.1).

Respiration measurements were taken 52 h after placement into treatment condition. These measurements were conducted at night between 21:00-00:00 h. To measure respiration, each sample was placed in a double-walled, lidded, metal thermos container with 400 mL of filtered seawater from its corresponding aquarium. Initial dissolved oxygen (DO) readings were conducted, containers were sealed, and a laboratory rotator (Laboratory Rotator Model G2, New Brunswick Scientific Co. Inc., New Brunswick, NJ, USA) was used to gently agitate the samples over a 1 h period. The laboratory rotator was set at its lowest setting (40 rpm) to disrupt the boundary layer around the tissue sample for realistic gas exchange rates while minimizing

mixing at the air-water interface. 1 h after initial DO readings, final DO readings were taken. All DO measurements were recorded using a YSI 556 Multiprobe System (YSI Inc., Yellow Springs, OH, USA) with randomized sampling order. Four control containers containing only seawater were included, to account for the interchange of oxygen between the air and water during the hour-long interval. Following respiration measurements all tissue samples were returned to their original tanks.

The third morning after sample collection, rapid light curves were generated fluorometrically (Junior PAM, Walz Germany) between 10:00-11:00 h to measure photosynthetic rates. Samples were removed from aquaria and placed in plastic containers in a uniform partial-light environment for approximately 10 min before initiating PAM measurements, individually, in random order. Once completed, all samples were dried in a drying oven at 60°C for five days and weighed. Dry weight was used to normalize respiration rates.

Through the duration of the experiment, the temperature, salinity, total alkalinity (TA), light, and pH in the aquaria were regularly monitored (Table 2.2 and 2.3) to assure consistency between trials and calculate pCO_2 using the CO_2sys software program (Pierrot, Lewis & Wallace, 2006). Temperature and light measurements were recorded every 30 min in each aquarium for the duration of the trial (HOBO Pendant Data Loggers, Onset, MA, USA). Several summary statistics of temperature (Table 2.4) were used to generate models in JMP software and results were compared to determine which would be most informative. The most useful metric for temperature was the mean temperature value from 2300-0200 h ("mean mid-night temp"), likely from low variance (Table 2.4).

To measure photosynthetically active radiation (PAR), a LI-COR Spherical Underwater Quantum Sensor (Lincoln, NE, USA) was deployed in the water bath holding the aquaria, at the same depth as the algal tissue. The sensor was attached to a data logger (LI-1400, LI-COR, Lincoln, NE, USA) that measured PAR every 30 min from 0630-1900 h for the duration of the trial. Due to researcher error, only two of the four trials were measured for PAR.

Salinity was measured in each aquarium twice per trial (YSI 556 Multiprobe System, calibrated with YSI Conductivity Calibrator Solution #3169).

pH was determined for each aquarium with a spectrophotometer (Molecular Devices SpectraMax M2, CA, USA) at the Hawai‘i Institute of Marine Biology (HIMB) twice per trial within three hours of seawater sample collection, using meta-cresol purple dye (Acrōs Organics, Thermo Fisher Scientific, Belgium) as an indicator (Clayton & Byrne, 1993, *in* Dickson, Sabine & Christian, 2007). For a detailed description of the spectrophotometric analysis methodology, see Appendix A.

For each aquarium, TA titrations were conducted using an automatic titrator (Titrino Plus 887, Metrohm AG, Switzerland, calibrated with YSI pH 4.00 and Oaklon pH 7.00 buffer solutions) at HIMB within 48 hours of seawater sample collection. For a detailed description of the alkalinity titration procedures, see Appendix B.

Respiration was calculated as oxygen consumed and converted to μmol O_2 g dw^{-1} h^{-1}. PAR exposure for each individual tank was calculated by experimentally deriving a site-specific equation to convert lux (HOBO logger unit of measurement) to PAR μmol photons m^{-2} s^{-1} (Appendix C). All logger light measurements were subsequently converted to PAR. Two calculations were employed to calculate PAR: (1) an average value of light measured between 0700-1730, and (2) the last PAR measurement taken before tissues were analyzed with Junior

PAM. pH values obtained by spectrophotometric analysis were converted to pCO_2 values using the CO_2sys software for Microsoft Excel (Pierrot, Lewis & Wallace, 2006).

Statistical Analysis

This study used a full-factorial, blocked design with randomly assigned treatments. Initially, the experimental unit was the header, the location of treatment assignment. Because each header fed two tanks, each containing individual thalli, these individuals could not be considered independent observations. However, during the analysis, headers were not shown to have a significant effect on any of the response variables (Tables 2.6-2.8), allowing individual tanks to be treated as experimental units. Thus, 32 samples were spread equally among four treatments, yielding a sample size of eight (n=8). Two samples died during the experiment and were excluded from analysis.

The experiment was blocked by week-long experimental trials. The eight tanks had fixed locations, but treatments were randomly assigned among the tanks at the start of each trial. Algae were haphazardly distributed into tanks after collection and cleaning. The order of respiration and PAM measurements was randomized for each trial. The order of salinity, temperature, pH, and total alkalinity measurements was not randomized.

Although an ANOVA had been planned for data analysis, seasonal changes in the ambient temperature of the seawater over the course of the experimental period led to an inability to maintain statistically significant temperature categories in the aquaria. As a result, generalized linear models were used to analyze data. All measured factors were treated as continuous variables and "Header" and "Tank" were evaluated as categorical variables. The three response variables measured and analyzed were respiration, maximum electron transport rate (ETR_{max}), and the minimum saturating irradiance (E_k). Each response variable was checked

for normality, homoscedasticity, and multivariate normality using graphical analysis of histograms and residual plots. For each response variable, a whole model was created which contained "Header" and "Tank" (nested in "Treatment") to ensure that neither header nor tank had a significant effect.

During the course of the experiment, mortality occurred in two samples. The first sample death may be linked to water stagnation from equipment failure within one aquarium. The second tissue died from unknown circumstances, despite the fact that all the parameters it was exposed to (temperature, pH, light) were duplicated in an adjacent tank and received water from the same header tank. It was therefore assumed that the mortality was not associated with factors being tested, but instead a factor inherent to the tissue itself (e.g. history prior to collection, recent injury). Both samples were excluded from all statistical analyses.

This study aimed to constrain pCO_2 levels at or below 940 μatm, the upper limit projected for year 2100 (Pörtner *et al.*, 2014). pCO_2 is difficult to measure continuously, thus pH was measured as a proxy for pCO_2, aiming to keep the pH values in the CO_2 treatments within 0.40 pH units from the pH of ambient seawater. However, three tissue samples experienced conditions outside of the established range (pCO_2 values of 1035, 1062, and 1232 μatm; pH values of 7.67, 7.66, and 7.61). Though these pCO_2 values were higher than desired, these values were retained in the analysis. *G. salicornia* is a dense, mound-forming alga capable of weaving thalli together so tightly that less than 1% of ambient irradiance penetrates to the bottom of the mound (Beach *et al.*, 1997; Martinez, Smith & Richmond, 2012). Within these mounds, thalli experience conditions different from ambient seawater, including significantly lower pH (Martinez, Smith & Richmond, 2012). Martinez and colleagues report a minimum pH value of 7.47 within a *G. salicornia* mound (Martinez, Smith & Richmond, 2012). If pH values within

mounds of *G. salicornia* are significantly lower than ambient seawater values, data that fall within these pH ranges are still considered ecologically significant.

Results

Respiration

Pooled respiration rates ranged from -5.03 to 15.89 μmol O_2 h^{-1} g^{-1} DW, with an average value of 6.64 and standard deviation 5.79 μmol O_2 h^{-1} g^{-1} DW. Respiration rates appeared to be influenced most strongly by the average mid-night temperatures and the square of the average mid-night temperatures. The complete model (Table 2.5) indicated that pCO_2 values did not have a significant effect on respiration values.

Photosynthesis: ETR_{max} and E_k

Pooled ETR_{max} values ranged from 24.72 to 85.40 μmol e^- m^{-2} s^{-1} (Table 2.6). The effects of mean mid-night temperature, pCO_2, and PAR (average between 0700-1730 h) were examined. The only factor in this model that had a significant effect was the pCO_2 treatment (Table 2.7). However, upon examining the residuals, three outlier data points were determined to have strong influence over the model (Figures 2.3 and 2.4). These outliers were extreme ETR_{max} values at extreme pCO_2 values and were included in the analysis, but it should be noted that when excluded, the pCO_2 factor ceased to be significant.

Pooled E_k values ranged from 164.1 to 715.1 μ mol photons m^{-2} s^{-1} (Table 2.6). The statistical model showed that the categorical variable "tank" (nested under "treatment") had a significant effect on E_k (Table 2.8). Therefore, whether the experimental treatments conditions or the confounding factor of "tank" was the dominant factor explaining E_k cannot be differentiated. Effect tests indicated that mean midnight temperature and the PAR measurement taken directly before Junior PAM measurements were significant factors. Data tables are included for reference

purposes, however, effects of treatment conditions cannot be determined as significant because of the significance of the tank as a confounding factor.

Discussion

Respiration and photosynthesis are two metabolic processes that contribute to the overall growth rate of a plant. From a mass balance perspective, Equation 2.1 (Carr, Duthie & Taylor, 1997) gives a simple model of plant growth:

$$\text{Equation 2.1:} \quad S = P - R - L$$

In this equation, S represents the change in standing stock of the plant (plant growth) as a function of the accumulation of biomass through gross photosynthesis (P), the loss of biomass through respiration (R) and other losses (L) (Carr, Duthie & Taylor, 1997). From this straightforward model, we can see that increasing photosynthesis contributes to positive growth and increasing respiration contributes to reductions in growth. Understanding how different climate change factors will affect these processes in *G. salicornia* can inform predictions of how successful this invasive alga will be in the next century.

Comparing results with other studies

Rates of respiration measured here were pooled and compared with those reported in Phooprong, Ogawa & Hayashizaki (2007). Respiration rates were measured for *G. salicornia* samples from three different locations (Okinawa, Japan; Rayong, Thailand; Phuket, Thailand) across a gradient of temperatures (Phooprong, Ogawa & Hayashizaki, 2007). Respiration rates from treatments at 25 °C and 30 °C, the temperatures closest to those in this study, are included in Figure 2.5 for comparison. Similar to the study presented here, temperature had a significant, positive effect on respiration rates (Phooprong, Ogawa & Hayashizaki, 2007). The respiration

rates found in this study were lower than the respiration rates found by Phooprong *et al.*, which may be tied to methodological differences. In this study, respiration rates were measured at night, several hours after any light exposure. In contrast, Phooprong *et al.* exposed plants to 15 min of darkness (zero irradiance for respiration measurements) before initiating DO measurements (Phooprong, Ogawa & Hayashizaki, 2007). A minimum of 30 min is considered an appropriate darkness interval to avoid increased respiration rates due to the phenomenon of light enhanced dark respiration - a period of elevated respiration rates directly following exposure to light (Florez-Sarasa *et al.*, 2012). Thus, higher respiration rates measured by Phooprong *et al.* may be attributable to light enhanced dark respiration resulting from an abbreviated dark period.

ETR_{max} values and E_k values from this study were pooled and compared with those from two previous experiments: Spafford, 2011 and Kawachi *et al.*, in prep. (Figures 2.6 and 2.7). Spafford's research examined photosynthetic parameters for *G. salicornia* at different photoacclimation states (high irradiance and low irradiance). The tissues collected by Spafford were orange (high irradiance acclimation) or red (low irradiance acclimation) expressing different pigment concentrations based on photoacclimation state (Beach *et al.*, 1997; Spafford, 2011). The tissue collected in this study and by Kawachi *et al.* was photoacclimated to intermediate irradiance and green in color (Kawachi M, pers. comm., 2017). These distinct photoacclimation states influence ETR_{max} and E_k values, with green tissue demonstrating the highest values and red tissue at the lowest values for both variables.

This result disagrees with earlier findings by Beach *et al.* (1997), comparing oxygen evolution light curves with in-vivo absorbance spectra in *G. salicornia* tissue acclimated to low irradiance (red color), intermediate irradiance (green color), and high irradiance (orange color).

For reference, P_{max} and I_k are conceptually similar to ETR_{max} and E_k, respectively, but the first pair is used to describe data obtained through oxygen evolution and the latter pair is used with data obtained through fluorometry. Maximum photosynthesis rate (P_{max}) values were negatively correlated with in-vivo absorbance peaks at wavelength 568 nm (associated with R-phycoerythrin) (Beach *et al.* 1997). Minimum saturating irradiance (I_k) values were positively correlated with peaks at wavelength 495 nm (assocated with R-phycoerythrin and carotenoids). Red tissue was associated with a high peak at 568 nm and low P_{max}, and a low peak at 495 nm and low I_k. Orange tissue showed the opposite tendencies: a low peak at 568 and high P_{max}, and a high peak at 495 and high I_k. Green tissue peaks were comparatively intermediate, indicating that P_{max} and I_k should be intermediate values relative to values from red and orange tissue. In this study, however, green tissue showed distinctly higher ETR_{max} and E_k values compared to red and orange tissue.

Temperature

Q_{10} studies posit that the rate of a process, such as photosynthesis, will increase with increasing temperature until an optimal thermal temperature is reached, after which the rate of the process will decrease rapidly (Davison, 1991; Laing, 2012). However, our research found no significant effects of temperature on ETR_{max}. The discrepancy may stem from the fact that changes associated with short-term exposure to temperatures seen in the Q_{10} studies do not necessarily reflect the physiology of an acclimated plant (Li, 1980; Davison, 1991). Other experiments which did allow algae to acclimate to temperature conditions found a correlation between lower growth temperature plants and higher maximum photosynthesis rates (Davison, 1991). Although an acclimation period of two days is not unprecedented (Lignell & Pedersén, 1989), it is possible that the samples did not fully acclimate to temperature conditions prior to

measurements. Another possibility is that our temperature range was not broad enough to produce a significant effect. Q_{10} studies often expose plants to a broad range of temperatures, with differences exceeding 20 °C between minimum and maximum temperatures (e.g. Phooprong, Ogawa & Hayashizaki, 2007). In contrast, the temperature range in this study was smaller (2 °C) to approximate climate change conditions within the next century.

The increase in respiration with increasing temperature that we observed aligns with the results of previous studies. Several studies that allow an acclimation period or grow the algae at specific temperature regimes have shown a link between increasing temperature and increasing dark respiration (Lapointe, Tenore & Dawes, 1984; Robarts & Zohary, 1987; Phooprong, Ogawa & Hayashizaki, 2007; Koch *et al.*, 2013). This increased respiration at higher temperatures is hypothesized to be the reason for the disconnect between optimal growth temperature and optimal photosynthesis temperatures (Li, 1980; Lapointe, Tenore & Dawes, 1984; Robarts & Zohary, 1987).

If 52 h was insufficient acclimation time, then the increased respiration rate observed in this study may reflect the increased cost of energy for acclimation to treatment conditions. The amount of time needed for an alga to acclimate to a different temperature regime is not well studied (Davison, 1991). There is evidence that some algae may not need acclimation periods at all so long as the new temperature treatment falls within a range of temperatures that do not cause temperature stress to the alga (Li, 1980; Lapointe, Tenore & Dawes, 1984). In one experiment, *Gracilaria tikvahiae* samples grown in an outdoor facility at 25-30 °C were transplanted into chambers and exposed to constant temperatures of 15, 20, 25, and 30 °C (Lapointe, Tenore & Dawes, 1984). Each sample's growth rate was monitored prior to and following the temperature change. Results indicated that the algal samples did not need time to

adjust to the temperature change; instead they continued to have exponential growth rates immediately after the transplantation. The sole exception was the algae transplanted into the 15 °C treatment, the thermal extreme for this clone of *Gracilaria tikvahiae*, which produced a lag of 5-9 days before the algae stabilized into steady-state growth (Lapointe, Tenore & Dawes, 1984). The temperatures experienced by algae in this experiment did not have a large range (22.24 °C minimum temperature and 29.65 °C maximum temperature observed over all trials) and were far from the thermal extremes of this alga (Smith *et al.*, 2004; Phooprong, Ogawa & Hayashizaki, 2007). Therefore, it seems probable that the increased respiration values observed are not caused by the increased energy cost of acclimation.

Carbon Dioxide

Prior to this study, at least three species in the genus *Gracilaria* showed increased growth with increased carbon dioxide availability (DeBusk & Ryther, 1984; Lignell & Pedersén, 1989; Xu, Zou & Gao, 2010). Although growth was not measured in this experiment, an increase in ETR_{max} was observed at higher pCO_2. Our results imply carbon limitation in *G. salicornia*, which would be consistent with the carbon limitation found in *G. tikvahiae* (DeBusk & Ryther, 1984), *G. segundata* (Lignell & Pedersén, 1989), and *G. lemaneiformis* (Xu, Zou & Gao, 2010). If *G. salicornia* is a carbon limited species, then climate change may increase the growth rate of this already invasive species (Harley *et al.*, 2012; Koch *et al.*, 2013). However, further testing is necessary to determine whether these potential increases in growth rate at higher pCO_2 values will be outweighed by accelerated respiration rates at higher temperatures, which could inhibit growth.

Most of the species studied in this genus and a majority of rhodophytes in general are able to use HCO_3^- via carbonic anhydrase (Lignell & Pedersén, 1989; Haglund *et al.*, 1992;

Andría *et al.*, 2001; Moulin *et al.*, 2011; Koch *et al.*, 2013). It would be imprudent to assume that *G. salicornia* can also use HCO_3^- solely based on the characteristics of other species; however, our results are reasonable if this assumption were true, given preferred use of CO_2 over HCO_3^- and the increased availability of CO_2 at lower pH values (Lignell & Pedersén, 1989; Haglund *et al.*, 1992). The different ways that algae acquire and use carbon are one cause of the contrasting, species-specific reactions that can occur when exposed to increased pCO_2 (Gao, Helbling & Häder, 2012; Koch *et al.*, 2013; Johnson, Price & Smith, 2014).

Potential future scenarios

There is evidence that *G. salicornia* in Hawai‘i will maintain or increase growth rates into the next century. The increase in ETR_{max} photosynthetic rates with increasing pCO_2 implies carbon limitation in *G. salicornia*. Carbon limitation in marine flora is the premise that the photosynthesis and growth rates of a specific species are not maximized at present-day dissolved inorganic carbon (DIC) concentrations (Koch *et al.*, 2013). Although we did not measure growth in *G. salicornia* directly, it has been shown for this alga that photosynthesis and growth rates are highly correlated (Smith *et al.* 2004). If *G. salicornia* is carbon limited, then future levels of increased pCO_2 will likely result in elevated growth rates (Harley *et al.*, 2012; Koch *et al.*, 2013) if the increase in respiration rates with warmer temperatures is low.

G. salicornia is known to grow in climates warmer than Hawai‘i (22 °C-30 °C), such as Okinawa, Japan (21 °C–32 °C) and along the coast of Thailand (25 °C–35 °C), which demonstrates that the species has the potential to thrive in warmer environments (Phooprong, Ogawa & Hayashizaki, 2007). If Hawai‘i sea surface maximum temperatures increase by 1-3 °C over the next century, *G. salicornia* will likely still be within its thermal limits, presuming that the genes contributing to thermal tolerance in the western Pacific population are present in the

Hawai‘i population as well. Genetic sequencing has indicated that the Hawai‘i population of *G. salicornia* is closely related to the Thailand population, with populations from Malaysia and the Philippines as out-groups, implying that the genetic capacity for thermal tolerance may be conserved (Yang, Geraldino & Kim, 2013).

High temperature events in Hawai‘i during 2014 and 2015 were associated with widespread coral bleaching across the state including Kāne‘ohe Bay and Hanauma Bay (Bahr, Jokiel & Rodgers, 2015; Rodgers *et al.*, 2017). However, these warming events may have affected invasive macroalgae as well. Invasive algae, primarily *Eucheuma denticulatum* and *Kappaphycus* alvarezii but also *Acanthophora spicifera* and *G. salicornia*, have established high abundance in Kāne‘ohe Bay since the 1990s and have been the target of focused removal efforts (Rodgers & Cox, 1999; Neilson *et al.*, in prep). Following the 2014/2015 warming events, researchers and managers noticed a drastic decrease in the abundance of *Eucheuma/ Kappaphycus* spp., but no noticeable decrease in *G. salicornia* abundance (Neilson BJ, pers. comm., 2017; Wall CB, pers. comm., 2017); however, further research is needed to address confounding factors, such as decreased trade wind velocity and decreased water flow, before temperature can be confirmed as the trigger for the algal decrease (Wall CB, pers. comm., 2017). Growth experiments indicate that *Eucheuma/ Kappaphycus* spp. have a lower thermal maximum than *G. salicornia* (Stimson J, unpublished data, 2017). Temperature-triggered changes in algal abundance and community structure have been demonstrated elsewhere for this specific warming event. Cox, Spalding & Foster reported a decline in native *Padina* and *Dictyota* spp. and a rise in invasive *Avrainvillea amadelpha* on the south shore of O‘ahu following the 2014 warming event (2017). Similar changes in algal abundance linked to temperature have been recorded in temperate climate zones, with high mortality in kelp after extreme temperature events (Martínez,

Cárdenas & Pinto, 2003; Wernberg *et al.*, 2016). The change of the Pacific Decadal Oscillation, a long-term and large-scale climate pattern affecting the North Pacific, from cool phase to warm phase may be one factor driving these higher temperatures now and into the next decades (Rodgers *et al.*, 2015). Together, these observations imply that in the next decades *G. salicornia* may have a competitive advantage over other invasive and native algae in warming waters, although more research is necessary.

Lastly, seawater changes within the next decades are expected to drastically decrease the abundance of coral on reefs (Hoegh-Guldberg *et al.*, 2007; Hughes *et al.* 2007; Kroeker, Micheli & Gambi, 2013; Pörtner *et al.*, 2014). On reefs, coral and algae compete for space and sunlight, and the increasing coral mortality rates may create space and rubble substrate in sunlit areas, offering the opportunity for algal expansion (McCook, Jompa & Diaz-Pulido, 2001; Hoegh-Guldberg, 2007; Hughes *et al.* 2007; Koch *et al.* 2013). The combination of an increased amount of habitat space, the thermal tolerance to survive in warming Hawaiian coastal areas through the next century, and the increase in carbon dioxide availability for this apparently carbon limited plant are all indications that *G. salicornia* will continue to be an invasive presence into the future.

The consequences of the sustained or increased abundance of *G. salicornia* on Hawai‘i’s reefs will include effects on several ecological levels. *G. salicornia* has shown the ability to compete successfully against native algal species that may occupy similar ecological niches (Russell, 1992). An increase in the abundance of *G. salicornia* may therefore increase the competition with similar native species such as *Laurencia nidifica* and *Gracilaria coronopifolia* (Russell, 1992). Increasing *G. salicornia* would lead to increased abundance in associated epiphytes and epifauna, which are distinct from the communities of epibionts on native algae

(Fukunaga, Peyton & Thomas, 2014). Overgrowth by *G. salicornia* creates harmful conditions for corals as the algal mounds significantly change the physical (sediment buildup and decreased irradiance) and chemical (lowered pH, periods of hypoxia and hyperoxia) conditions beneath them (Martinez, 2012; Martinez, Smith & Richmond, 2012). Additionally, presence of *G. salicornia* led to decreased survivorship in coral larvae in lab experiments, an effect that would decrease coral ability to recolonize algal dominated reefs, though these results were not replicated in field studies (Martinez, 2012). Decreased abundance of native algal species, changes in invertebrate abundances, and further deterioration of corals are some of the effects that can be expected with *G. salicornia*'s abundance increase.

Conclusion and future studies

In Hawai‘i, *G. salicornia* is one of several macroalgae that may invade and overgrow coral reefs within the next century. Understanding how pCO_2 and temperature each affect photosynthesis and respiration can help us to understand how the alga reacts to these factors at a physiological level. In this experiment, it was found that respiration increases with increasing temperature and photosynthesis increases with increasing pCO_2. The increase in photosynthesis rates may be indicative of carbon limitation at current DIC concentrations, which would mean increased growth with increased pCO_2. Meta-analyses from multiple studies may show patterns in how different factors and combinations of factors affect macroalgae at the physiological and community level (Johnson, Price & Smith, 2014).

There are a number of gaps to fill for future studies. A direct step after this research would be a similar study that incorporates a growth measurement of *G. salicornia*, with a longer acclimation and trial period. Because interactive effects of pCO_2 and temperature were not significant for this alga, the full-factorial experimental design could be exchanged for two single-

factor concurrent experiments. This change would allow for a greater number of replicates per experimental trial.

Recent occurrences including the 2014/2015 warming events and subsequent decrease in *Eucheuma/Kappaphycus* spp. abundance (Neilson BJ, pers. comm., 2017; Wall CB, pers. comm., 2017) and the increased green sea turtle foraging behavior (Bahr *et al.*, in prep) merit further investigation. Currently, manual alien algae removal efforts in Kāneʻohe Bay by the State of Hawaiʻi Department of Aquatic Resources focus specifically on species of *Eucheuma and Kappaphycus*. Further, abundance of *G. salicornia* decreases as an effect of sea urchin out-planting (Neilson *et al.*, in prep). If growth by species of *Eucheuma or Kappaphycus* is more temperature sensitive than growth of *G. salicornia*, as has been observed in the field, this could have important repercussions for management (Neilson BJ, pers. comm., 2017; Wall CB, pers. comm., 2017). If the next decades in Hawaiʻi bring high temperatures as predicted from both global climate change and the Pacific Decadal Oscillation warm phase (Rodgers *et al.* 2015), then a shift in priority from species of *Eucheuma* and *Kappaphycus* to *G. salicornia* and other invasive algae may be important.

Climate change will occur gradually over decades, and long-term (months to years) studies on how this will affect macroalgae are lacking. Immediate changes in algal physiology when exposed to a treatment are not identical to long-term changes, when the algae have had a chance to acclimate (Li, 1980; Davison, 1991) and week-long experiments may not capture the full range of effects that treatments will have over time. Long-term studies might even include periods of reproduction, to examine the effect of treatment on multiple life stages. Replicating a community in a mesocosm, with corals, macroalgae, crustose coralline algae, and invertebrates

in long-term manipulative experiments is ideal in ecological climate change research, but even without those resources, individual linkages can be examined.

Literature Cited

Ampou EE, Johan O, Menkes CE, Niño F, Birol F, Ouillon S, Andréfouët S. 2017. Coral mortality induced by the 2015-2016 El-Niño in Indonesia: the effect of rapid sea level fall. *Biogeosciences* 14:817-826.

Andría JR, Brun FG, Pérez-LLoréns JL, Vergara JJ. 2001. Acclimation responses of *Gracilaria* sp. (Rhodophyta) and *Enteromorpha intestinalis* (Chlorophyta) to changes in the external inorganic carbon concentration. *Botanica Marina* 44:361–370. DOI: 10.1515/BOT.2001.046.

Bahr KD, Coffey DM, Rodgers K, Balazs GH. In prep. Green sea turtles on the move for food: *Honu* in Hawai‘i grazing their way into new habitats.

Bahr KD, Jokiel PL, Rodgers KS. 2015. The 2014 coral bleaching and freshwater flood events in Kāne‘ohe Bay, Hawai‘i. *PeerJ* 3:e1136. DOI: 10.7717/peerj.1136

Beach KS, Borgeas HB, Nishimura NJ, Smith CM. 1997. *In vivo* absorbance spectra and the ecophysiology of reef macroalgae. *Coral Reefs* 16:21-28.

Carr GM, Duthie HC, Taylor WD. 1997. Models of aquatic plant productivity: a review of the factors that influence growth. *Aquatic Botany* 59:195-215.

Cesar H, van Beukering P, Pintz S, Dierking J. 2002. Economic valuation of the coral reefs of Hawai‘i: final report. Report to: University of Hawai‘i for the Hawai‘i Coral Reef Initiative Research Program. *Available at* https://www.coris.noaa.gov/portals/pdfs/hicesar.pdf (Accessed 20 June 2017). pp1-123.

Clayton TD, Byrne RH. 1993. Spectrophotometric seawater pH measurements: total hydrogen ion concentration scale calibration of m-cresol purple and at-sea results. *Deep Sea Research Part I: Oceanographic Research Papers* 40:2115-2129.

Cox TE, Spalding HL, Foster MS. 2017. Spatial and temporal variation of diverse inter-tidal algal assemblages in southwest O‘ahu. *Marine Ecology* 38:e12429. DOI: 10.1111/maec.12429.

Davison IR. 1991. Environmental effects on algal photosynthesis: temperature. *Journal of Phycology* 27:2-8. DOI: 10.1111/j.0022-3646.1991.00002.x.

DeCarlo TM, Cohen AL, Wong GT, Davis KA, Lohmann P, Soong K. 2017. Mass coral mortality under local amplification of 2° C ocean warming. *Scientific Reports* 7:44586.

DeBusk T, Ryther J. 1984. Effects of seawater exchange, pH and carbon supply on the growth of *Gracilaria tikvahiae* (Rhodophyceae) in large-scale cultures. *Botanica Marina* 27:357-362.

Diaz-Pulido G, Gouezo M, Tilbrook B, Dove S, Anthony KRN. 2011. High CO_2 enhances the competitive strength of seaweeds over corals. *Ecology Letters* 14:156–162. DOI: 10.1111/j.1461-0248.2010.01565.x.

Dickson AG, Sabine CL, and Christian JR, eds. 2007. Guide to best practices for ocean CO_2 measurements. *PICES Special Publication* 3:1-191.

Doney SC, Fabry VJ, Feely RA, Kleypas JA. 2009. Ocean acidification: The other CO_2 problem. *Annual Review of Marine Science* 1:169–192. DOI: 10.1146/annurev.marine.010908.163834.

Enochs IC, Manzello DP, Donham EM, Kolodziej G, Okano R, Johnston L, Young C, Iguel J, Edwards CB, Fox MD, Valentino L, Johnson S, Benavente D, Clark SJ, Carlton R, Burton T, Eynaud Y, Price NN. 2015. Shift from coral to macroalgae dominance on a volcanically acidified reef. *Nature Climate Change* 5:1083-1089. DOI: 10.1038/nclimate2758.

Florez-Sarasa I, Araújo WL, Wallström SV, Rasmusson AG, Fernie AR, Ribas-Carbo M. 2012. Light-responsive metabolite and transcript levels are maintained following a dark-adaptation period in leaves of Arabidopsis thaliana. *New Phytologist* 195:136-148.

Fukunaga A, Peyton KA, Thomas FIM. 2014. Epifaunal community structure and ammonium uptake compared for the invasive algae, *Gracilaria salicornia* and *Acanthophora spicifera*, and the native alga, *Padina thivyi*. *Journal of Experimental Marine Biology and Ecology* 456:78-86.

Gao K, Helbling EW, Häder DP. 2012. Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming. *Marine Ecology Progress Series* 470:167-189. DOI: 0.3354/meps10043

Godwin S, Rodgers K, Jokiel PL. 2006. Reducing potential impact of invasive marine species in the Northwestern Hawaiian Islands marine national monument. Report to: Northwest Hawaiian Islands Marine National Monument Administration. no. DOI, NOAA, National Ocean Service MOA 2005-008/6882Amendment No. 001. *Available at* http://www.academia.edu/13857287/Reducing_potential_impact_of_invasive_marine_species_in_the_northwestern_hawaiian_islands_marine_national_monument (Accessed 6 June 2017). pp1-66.

Haglund K, Björk M, Ramazanov Z, García-Reina G, Pedersén M. 1992. Role of carbonic anhydrase in photosynthesis and inorganic-carbon assimilation in the red alga *Gracilaria tenuistipitata*. *Planta* 187:1–8. DOI: 10.1007/BF00201951.

Harley CDG, Anderson KM, Demes KW, Jorve JP, Kordas RL, Coyle TA, Graham MH. 2012. Effects of climate change on global seaweed communities. *Journal of Phycology* 48:1064–1078. DOI: 10.1111/j.1529-8817.2012.01224.x.

Ho M, Carpenter RC. 2017. Differential growth responses to water flow and reduced pH in tropical marine macroalgae. *Journal of Experimental Marine Biology and Ecology* 491:58–65. DOI: 10.1016/j.jembe.2017.03.009.

Hoegh-Guldberg O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research* 50:839–866. DOI: 10.1071/mf99078.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME. 2007. Coral reefs under rapid climate change and ocean acidification. *Science* 318:1737–1742. DOI: 10.1126/science.1152509.

Hughes TP, Kerry JT, Álvarez-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH, Babcock RC, Beger M, Bellwood DR, Berkelmans R, Bridge TC. 2017. Global warming and recurrent mass bleaching of corals. *Nature* 543:373-7. DOI: 10.1038/nature21707.

Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschaniwskyj N, Pratchett MS, Steneck RS, Willis B. 2007. Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Current Biology* 17:360–365. DOI: 10.1016/j.cub.2006.12.049.

Israel A, Hophy M. 2002. Growth, photosynthetic properties and Rubisco activities and amounts of marine macroalgae grown under current and elevated seawater CO_2 concentrations. *Global Change Biology* 8:831–840. DOI: 10.1046/j.1365-2486.2002.00518.x.

Johnson MD, Price NN, Smith JE. 2014. Contrasting effects of ocean acidification on tropical fleshy and calcareous algae. *PeerJ* 2:e411. DOI: 10.7717/peerj.411.

Jokiel PL, Bahr KD, Rodgers KS. 2014. Low-cost, high-flow mesocosm system for simulating ocean acidification with CO_2 gas. *Limnology and Oceanography: Methods* 12:313–322. DOI: 10.4319/lom.2014.12.313.

Jokiel PL, Brown EK. 2004. Global warming, regional trends and inshore environmental conditions influence coral bleaching in Hawai‘i. *Global Change Biology* 10:1627–1641. DOI: 10.1111/j.1365-2486.2004.00836.x.

Kawachi MS, Van Houtan KS, Smith CM. In prep. Elevated nutrients promote growth of coral reef macroalgae in Hawai‘i.

Koch M, Bowes G, Ross C, Zhang XH. 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology* 19:103–132. DOI: 10.1111/j.1365-2486.2012.02791.x.

Kram SL, Price NN, Donham EM, Johnson MD, Kelly ELA, Hamilton SL, Smith JE. 2016. Variable responses of temperate calcified and fleshy macroalgae to elevated pCO_2 and warming. *ICES Journal of Marine Science* 73:693–703. DOI: 10.1093/icesjms/fsv168.

Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso JP. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology* 19:1884-1896. DOI: 10.1111/gcb.12179.

Kroeker KJ, Micheli F, Gambi MC. 2013. Ocean acidification causes ecosystem shifts via altered competitive interactions. *Nature Climate Change* 3:156-159.

Laing WA 2012. Temperature and light response curves for photosynthesis in kiwifruit (*Actinidia chinensis*) cv. Hayward. *New Zealand Journal of Agricultural Research* 28:117–124. DOI: 10.1080/00288233.1985.10427004.

Lapointe BE, Tenore KR, Dawes CJ. 1984. Interactions between light and temperature on the physiological ecology of *Gracilaria tikvahiae* (Gigartinales: Rhodophyta). *Marine Biology* 80:161–170. DOI: 10.1007/BF02180183.

Li W. 1980. Temperature adaptation in phytoplankton: Cellular and photosynthetic characteristics. In: Falkowski P, ed. *Primary Productivity in the Sea*. Springer US, pp. 259-277

Lignell Å, Pedersén M. 1989. Effects of pH and inorganic carbon concentration on growth of *Gracilaria secundata*. *British Phycological Journal*. DOI: 10.1080/00071618900650071.

Martínez EA, Cárdenas L, Pinto R. 2003. Recovery and genetic diversity of the intertidal kelp *Lessonia nigrescens* (Phaeophyceae) 20 years after El Niño 1982/831. *Journal of Phycology* 39:504-508.

Martinez JA. 2012. The physical and physiological impacts of the invasive red macroalga *Gracilaria salicornia* Dawson to coral reef habitat quality and coral health. *Ph. D. Dissertation, University of Hawai'i at Mānoa*, pp. 1-127.

Martinez JA, Smith CM, Richmond RH. 2012. Invasive algal mats degrade coral reef physical habitat quality. *Estuarine, Coastal and Shelf Science* 99:42-49.

McCook L, Jompa J, Diaz-Pulido G. 2001. Competition between corals and algae on coral reefs: a review of evidence and mechanisms. *Coral Reefs* 19:400–417. DOI: 10.1007/s003380000129.

Moulin P, Andría JR, Axelsson L, Mercado JM. 2011. Different mechanisms of inorganic carbon acquisition in red macroalgae (Rhodophyta) revealed by the use of TRIS buffer. *Aquatic botany* 95:31-38.

Neilson BJ, Wall CB, Mancini FT, Gewecke CA. in prep. Herbivore biocontrol and manual removal successfully reduce invasive macroalgae on coral reefs.

Nelson SG, Glenn EP, Moore D, Ambrose B. 2009. Growth and distribution of the macroalgae *Gracilaria salicornia* and *G. parvispora* (Rhodophyta) established from aquaculture introductions at Molokaʻi, Hawaiʻi. *Pacific Science* 63:383–396. DOI: 10.2984/049.063.0307.

Phooprong S, Ogawa H, Hayashizaki K. 2007. Photosynthetic and respiratory responses of *Gracilaria salicornia* (C. Ag.) Dawson (Gracilariales, Rhodophyta) from Thailand and Japan. *Journal of Applied Phycology* 19:795–801. DOI: 10.1007/s10811-007-9194-3.

Pierrot D, Lewis E, Wallace DWR. 2006. *MS Excel Program Developed for CO_2 System Calculations*. Oak Ridge, Tennessee: Carbon dioxide information analysis center, Oak Ridge National Laboratory, U.S. Department of Energy.

Pörtner H, Karl D, Boyd P, Cheung W, Lluch-Cota S, Nojiri Y, Schmidt D, Zavialov P. 2014. Ocean Systems. In: Field C, Barros V, Dokken D, Mach K, Mastrandrea M, Bilir T, Chatterjee M, Ebi K, Estrada Y, Genova R *et al. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge and New York: Cambridge University Press, pp. 411-484.

Porzio L, Buia MC, Hall-Spencer JM. 2011. Effects of ocean acidification on macroalgal communities. *Journal of Experimental Marine Biology and Ecology* 400:278–287. DOI: 10.1594/PANGAEA.728715.

Robarts RD, Zohary T. 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *New Zealand Journal of Marine and Freshwater Research* 21:391–399. DOI: 10.1080/00288330.1987.9516235.

Rodgers KS, Bahr KD, Jokiel PJ, Richards Donà A. 2017. Patterns of bleaching and mortality following widespread warming events in 2014 and 2015 at the Hanauma Bay Nature Preserve, Hawaiʻi. *Peerj* .DOI: 10.7717/peerj.3355

Rodgers KS, Cox EF. 1999. Rate of spread of introduced rhodophytes *Kappaphycus alvarezii, Kappaphycus striatum*, and *Gracilaria salicornia* and their current distributions in Kāneʻohe bay, Oʻahu, Hawaiʻi. *Pacific Science* 53:232–241.

Rodgers KS, Jokiel PL, Brown EK, Hau S, Sparks R. 2015. Over a decade of change in spatial and temporal dynamics of Hawaiian coral reef communities. *Pacific Science* 69:1-13.

Russell DJ. 1992. The ecological invasion of Hawaiian reefs by two marine red algae, *Acanthophora spicifera* (Vahl) Boerg. and *Hypnea musciformis* (Wulfen) J. Ag., and their association with two native species, *Laurencia nidifica* J. Ag. and *Hypnea cervicornis* J. Ag. *ICES Marine Science Symposium* 194:110-125.

Smith JE, Hunter CL, Conklin EJ, Most R, Sauvage T, Squair C, Smith CM. 2004. Ecology of the invasive red alga *Gracilaria salicornia* (Rhodophyta) on Oʻahu, Hawaiʻi. *Pacific*

Science 58:325–343.

Smith JE, Hunter CL, Smith CM. 2002. Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian Islands. *Pacific Science* 56:299–315. DOI: 10.1017/CBO9780511751790.021.

Spafford D. 2011. The physiology of photoacclimation and photoprotection in the invasive red alga, *Gracilaria salicornia*. *M.S. thesis, University of Hawai'i at Mānoa*, pp. 1-113.

The National Weather Service Forecast Office, Honolulu HI, 2014. Observed weather reports. *Available at http://w2.weather.gov/climate/index.php?wfo=hnl*(accessed 27 January 2017)

Wernberg T, Bennett S, Babcock RC, de Bettignies T, Cure K, Depczynski M, Dufois F, Fromont J, Fulton CJ, Hovey RK, Harvey ES. 2016. Climate-driven regime shift of a temperate marine ecosystem. *Science* 353:169-172.

Wernberg T, Smale DA, Thomsen MS. 2012. A decade of climate change experiments on marine organisms: procedures, patterns and problems. *Global Change Biology* 18:1491–1498. DOI: 10.1111/j.1365-2486.2012.02656.x.

Xu Z, Zou D, Gao K. 2010. Effects of elevated CO_2 and phosphorus supply on growth, photosynthesis and nutrient uptake in the marine macroalga *Gracilaria lemaneiformis* (Rhodophyta). *Botanica Marina* 53:123-129.

Yang M, Geraldino Y, Kim P. 2013. DNA barcode assessment of *Gracilaria salicornia* (Gracilariaceae, Rhodophyta) from Southeast Asia. *Botanical Studies* 54:1-9.

Table 2.1: Timeline and weather conditions for the experimental periods from the National Weather Service Forecast Office, Honolulu International Airport weather station (located ~5 km from experiment location).

Trial number	Trial Dates (year 2016)	Max Temp (°C)	Min Temp (°C)	Average day length (hr:min)	Total rainfall during trial (cm)
1	Oct. 17-20	30.6	23.9	11:37	0.0762
2	Oct. 31- Nov. 3	29.4	22.2	11:21	0
3	Nov. 7-10	29.4	18.9	11:14	0
4	Nov. 28- Dec. 1	27.8	21.1	10:57	0.4572

Table 2.2: Summary of measured parameters, frequency, and instruments

Measured parameter	**Frequency of measurement during trial period**	**Measurement Instrument**
Dissolved oxygen	Once, for respiration rate determination	YSI 556 Multiprobe System
Temperature	Every 30 min	HOBO data logger
Salinity	Once per trial	YSI 556 Multiprobe System
Total Alkalinity	Twice per trial	Titrino Plus 877, Metrohm
Electron Transfer Rate	Once, for photosynthetic rate determination	Junior PAM, Walz Germany
pH	Twice per trial	Spectrophotometer

Table 2.3: Temperature summary statistics comparisons

Summary statistic	Average (°C)	Standard deviation (°C)
Average of all temperature values for each sample	26.78	1.08
Average of temperature values from 1100-1400 h for each sample	27.83	1.08
Average of temperature values from 2300-0200 h for each sample	26.43	1.07

Table 2.4: Means of measured factors by treatment

Treatment category	**Mean midnight temperature (°C)**		**pH**		**Salinity (ppt)**		**Total Alkalinity (equivalents)**		**PAR average 0700-1730 (µmol photons m^{-2} s^{-1})**		**pCO_2 values (µatm)**	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
ambient temp, $+CO_2$	26.14	1.09	7.81	0.10	34.44	0.17	2274.66	29.26	101.9	47.4	758	203
ambient temp, normal pH	26.31	1.32	8.04	0.01	34.52	0.05	2285.49	7.46	101.6	36.6	392	13
increased temp, $+CO_2$	26.54	1.05	7.81	0.11	34.53	0.08	2286.69	7.18	87.9	25.1	749	234
increased temp, normal pH	26.72	0.94	8.03	0.01	34.52	0.07	2285.68	6.81	99.5	38.0	402	17

Table 2.5: Multiple regression model analysis (effect tests and parameter estimates) of respiration rates ($R^2 = 0.74$). Values significant at the level of $\alpha = 0.05$ are marked with an asterisk (*).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
pCO2 values	1	1	54.76966	2.3823	0.1510
Treatment_Cat[Tank]	12	12	392.64153	1.4232	0.2832
Header	3	3	81.77512	1.1857	0.3598
mean mid night temp	1	1	159.46045	6.9361	0.0233*
mean mid night temp*mean mid night temp	1	1	143.98345	6.2629	0.0294*

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-100.6568	36.45083	-2.76	0.0185*
pCO2 values	0.0164813	0.010678	1.54	0.1510
Tank[1]:Treatment_Cat[cool, CO2]	-4.391973	4.276425	-1.03	0.3265
Tank[2]:Treatment_Cat[cool, CO2]	12.366235	5.369305	2.30	0.0418*
Tank[2]:Treatment_Cat[cool, norm]	-6.162523	6.853204	-0.90	0.3878
Tank[2]:Treatment_Cat[hot, CO2]	-2.259006	4.677362	-0.48	0.6386
Tank[3]:Treatment_Cat[cool, norm]	-8.170384	4.48763	-1.82	0.0959
Tank[4]:Treatment_Cat[cool, CO2]	4.4410629	3.297811	1.35	0.2052
Tank[5]:Treatment_Cat[cool, CO2]	-3.224216	5.031248	-0.64	0.5348
Tank[5]:Treatment_Cat[hot, CO2]	0.2386172	5.174219	0.05	0.9640
Tank[6]:Treatment_Cat[cool, CO2]	2.5294539	4.118306	0.61	0.5516
Tank[7]:Treatment_Cat[cool, norm]	-10.08822	4.813103	-2.10	0.0600
Tank[8]:Treatment_Cat[cool, CO2]	-0.662496	5.473208	-0.12	0.9058
Tank[8]:Treatment_Cat[cool, norm]	0.3322521	5.539338	0.06	0.9532
Header[1]	-4.962094	3.082751	-1.61	0.1358
Header[2]	0.5013599	3.355918	0.15	0.8839
Header[3]	-1.786201	4.031497	-0.44	0.6663
mean mid night temp	3.5788475	1.358893	2.63	0.0233*
(mean mid night temp-26.4265)*(mean mid night temp-26.4265)	3.9358068	1.5727	2.50	0.0294*

Table 2.6: Summary of pooled ETR_{max}, and E_k values.

	Mean	**St. Dev.**	**Min.**	**Max.**
ETR_{max} ($\mu mol\ e^{-}\ m^{-2}\ s^{-1}$)	47.31	14.15	24.72	85.40
E_k ($\mu mol\ photons\ m^{-2}\ s^{-1}$)	367.3	144.3	164.1	715.1

Table 2.7: Multiple regression model analysis (effect tests and parameter estimates) of ETR_{max} values ($R^2 = 0.77$). Values significant at the level of $\alpha = 0.05$ are marked with an asterisk (*).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
mean mid night temp	1	1	272.71249	2.2943	0.1580
pCO2 values	1	1	674.08391	5.6711	0.0364*
PAR avg 700-1730	1	1	42.73079	0.3595	0.5609
Treatment_Cat[Tank]	12	12	979.35794	0.6866	0.7362
Header	3	3	381.54817	1.0700	0.4014

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-93.36209	72.48884	-1.29	0.2242
mean mid night temp	4.3517836	2.873026	1.51	0.1580
pCO2 values	0.0526896	0.022125	2.38	0.0364*
PAR avg 700-1730	-0.041859	0.069815	-0.60	0.5609
Tank[1]:Treatment_Cat[cool, CO2]	15.04281	9.732353	1.55	0.1505
Tank[2]:Treatment_Cat[cool, CO2]	-11.11109	12.77084	-0.87	0.4029
Tank[2]:Treatment_Cat[cool, norm]	15.659065	15.00714	1.04	0.3191
Tank[2]:Treatment_Cat[hot, CO2]	-7.318108	10.53774	-0.69	0.5018
Tank[3]:Treatment_Cat[cool, norm]	2.0966482	10.43099	0.20	0.8444
Tank[4]:Treatment_Cat[cool, CO2]	-2.197423	7.185643	-0.31	0.7655
Tank[5]:Treatment_Cat[cool, CO2]	17.120559	11.35435	1.51	0.1598
Tank[5]:Treatment_Cat[hot, CO2]	1.0390469	11.85469	0.09	0.9317
Tank[6]:Treatment_Cat[cool, CO2]	7.6644968	9.558157	0.80	0.4396
Tank[7]:Treatment_Cat[cool, norm]	-0.445841	10.21297	-0.04	0.9660
Tank[8]:Treatment_Cat[cool, CO2]	-5.365603	10.35979	-0.52	0.6148
Tank[8]:Treatment_Cat[cool, norm]	5.4047044	11.46578	0.47	0.6466
Header[1]	-6.153538	6.922712	-0.89	0.3931
Header[2]	-13.84851	7.769408	-1.78	0.1023
Header[3]	12.05755	9.590788	1.26	0.2347

Table 2.8: Multiple regression model analysis (effect tests and parameter estimates) of E_k values ($R^2 = 0.93$). Values significant at the level of $\alpha = 0.05$ are marked with an asterisk (*).

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
mean mid night temp	1	1	19298.08	4.8601	0.0497*
pCO2 values	1	1	7651.21	1.9269	0.1926
Treatment_Cat[Tank]	12	12	211361.41	4.4358	0.0097*
PAR before PAM	1	1	27812.03	7.0043	0.0227*
Header	3	3	14425.02	1.2110	0.3514

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	1489.7906	511.3846	2.91	0.0141*
mean mid night temp	-39.72275	18.01841	-2.20	0.0497*
pCO2 values	0.1969359	0.141871	1.39	0.1926
Tank[1]:Treatment_Cat[cool, CO2]	116.20585	54.94273	2.12	0.0581
Tank[2]:Treatment_Cat[cool, CO2]	-136.3667	73.73894	-1.85	0.0914
Tank[2]:Treatment_Cat[cool, norm]	55.494449	83.9632	0.66	0.5223
Tank[2]:Treatment_Cat[hot, CO2]	-169.7469	62.08165	-2.73	0.0194*
Tank[3]:Treatment_Cat[cool, norm]	5.8186821	57.69482	0.10	0.9215
Tank[4]:Treatment_Cat[cool, CO2]	-123.0186	40.63728	-3.03	0.0115*
Tank[5]:Treatment_Cat[cool, CO2]	41.366339	65.39677	0.63	0.5400
Tank[5]:Treatment_Cat[hot, CO2]	18.160414	69.66733	0.26	0.7992
Tank[6]:Treatment_Cat[cool, CO2]	-64.93524	55.07522	-1.18	0.2633
Tank[7]:Treatment_Cat[cool, norm]	12.516165	58.32975	0.21	0.8340
Tank[8]:Treatment_Cat[cool, CO2]	292.52723	62.46509	4.68	0.0007*
Tank[8]:Treatment_Cat[cool, norm]	-164.3833	68.27046	-2.41	0.0347*
PAR before PAM	-6.526965	2.466204	-2.65	0.0227*
Header[1]	2.0116702	42.75776	0.05	0.9633
Header[2]	-42.73803	44.4315	-0.96	0.3568
Header[3]	-26.80649	52.92359	-0.51	0.6225

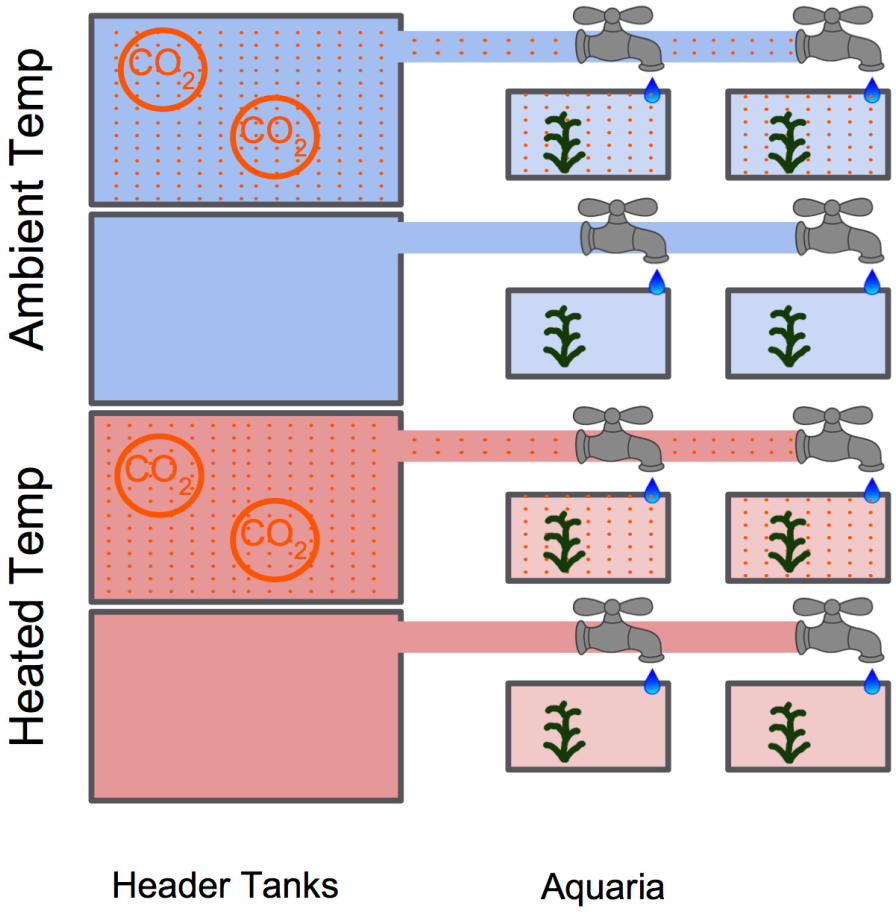

Figure 2.1: Representation of tank set-up. Seawater from Honolulu harbor is pumped into the header tanks where treatments (heat/no heat; CO_2/no CO_2) were applied. Water flowed from the elevated headers into eight aquaria (two per header) each containing an algal sample. Actual aquarium positions were randomized in each trial.

Figure 2.2: Photograph of tank set-up at Ānuenue Fisheries Research Center. Seawater flows continuously from elevated header tanks through PVC pipes and plastic tubing into individual aquaria. Wooden scaffolds keep the plastic tubes submerged so seawater flows in from the bottom, ensuring mixing. Excess water overflows from the top of the aquaria into larger water baths.

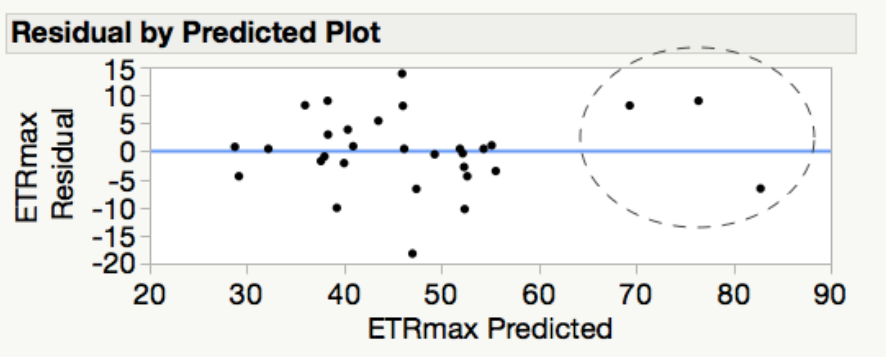

Figure 2.3: Graph of the residuals by predicted values for the ETR_{max} whole model. Blue line indicates the linear regression of the model. Visual outliers are indicated within dotted circle.

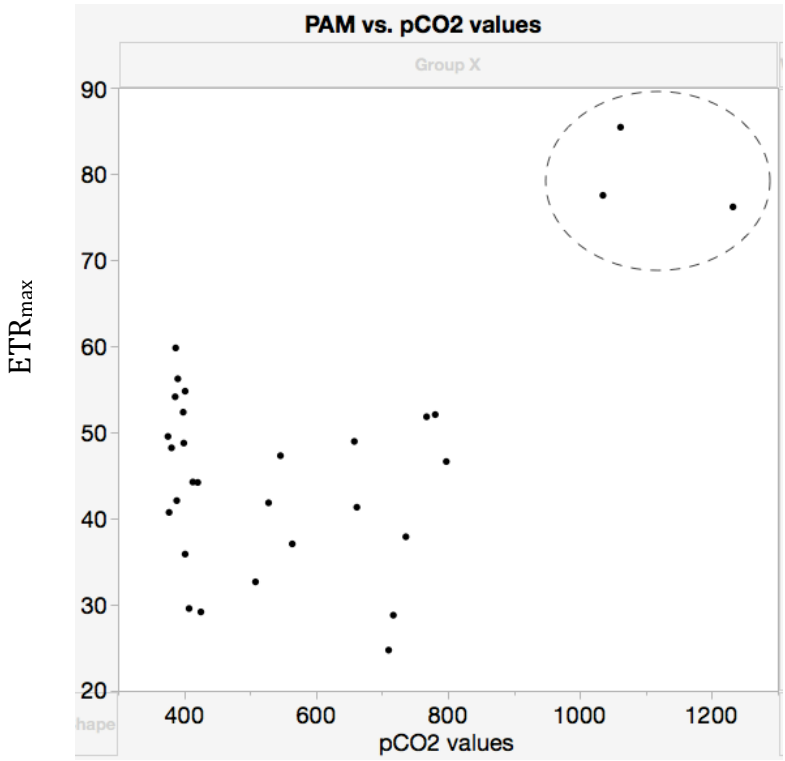

Figure 2.4: ETR_{max} plotted against pCO_2 values (µmol electron $m^{-2}s^{-1}$ and µatm, respectively). The same three outliers as in Figure 2.3 are indicated by the dotted circle.

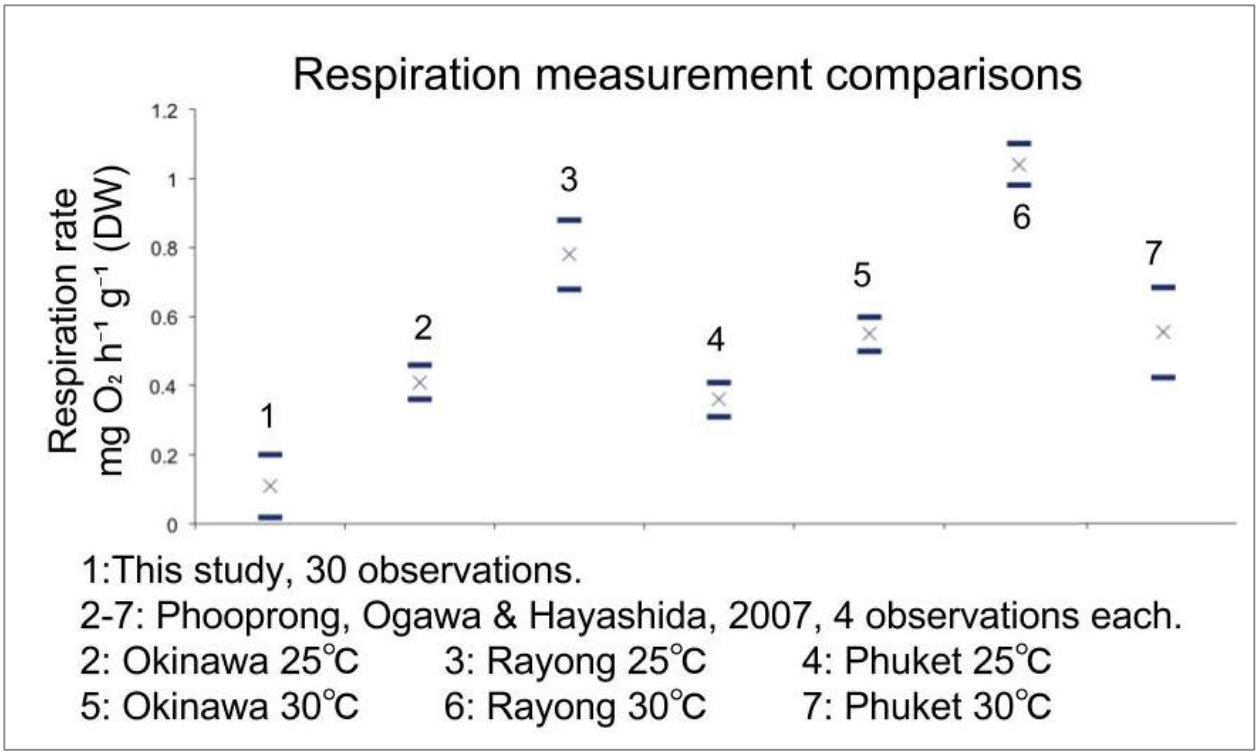

Figure 2.5: Comparison of respiration rates. Means (x) and standard deviations (bars) of respiration rates from this study and Phooprong, Ogawa & Hayashida (2007).

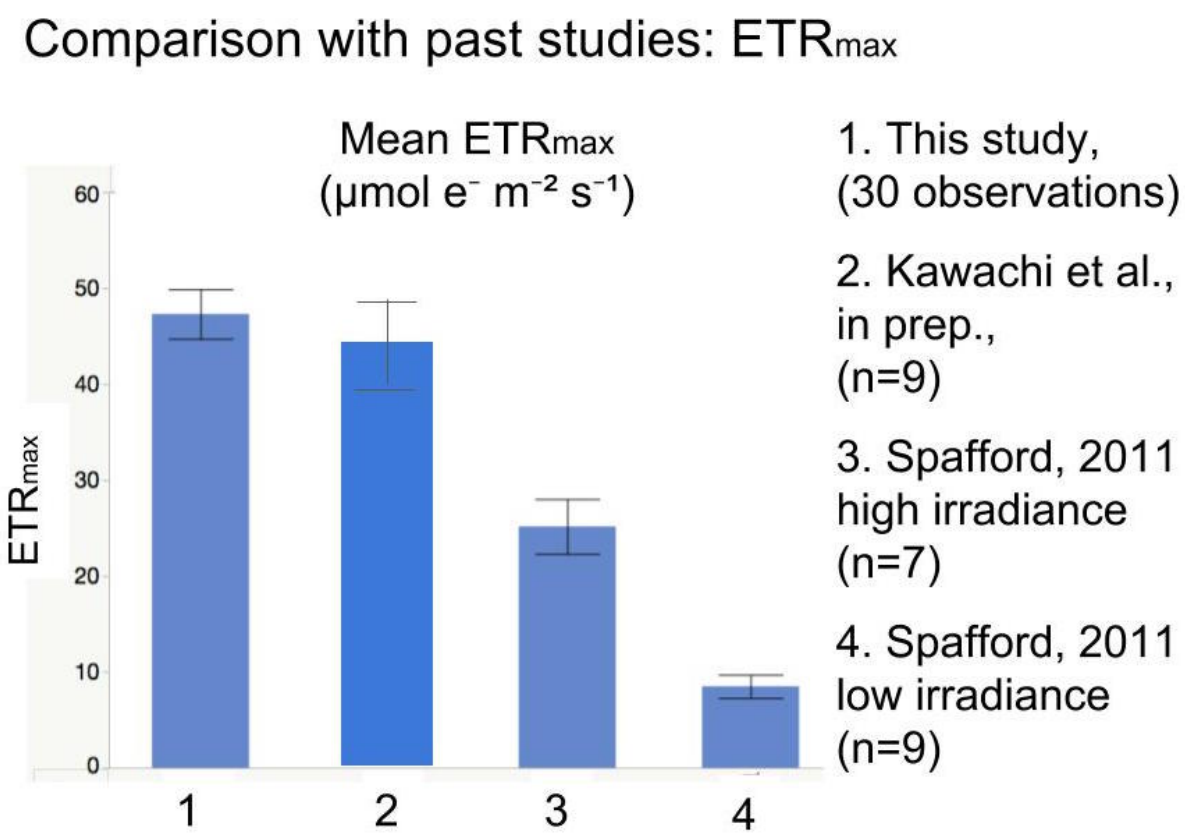

Figure 2.6: Comparison of ETR_{max}. Means and standard errors shown are from this study, Kawachi *et al.*, in prep and Spafford (2011).

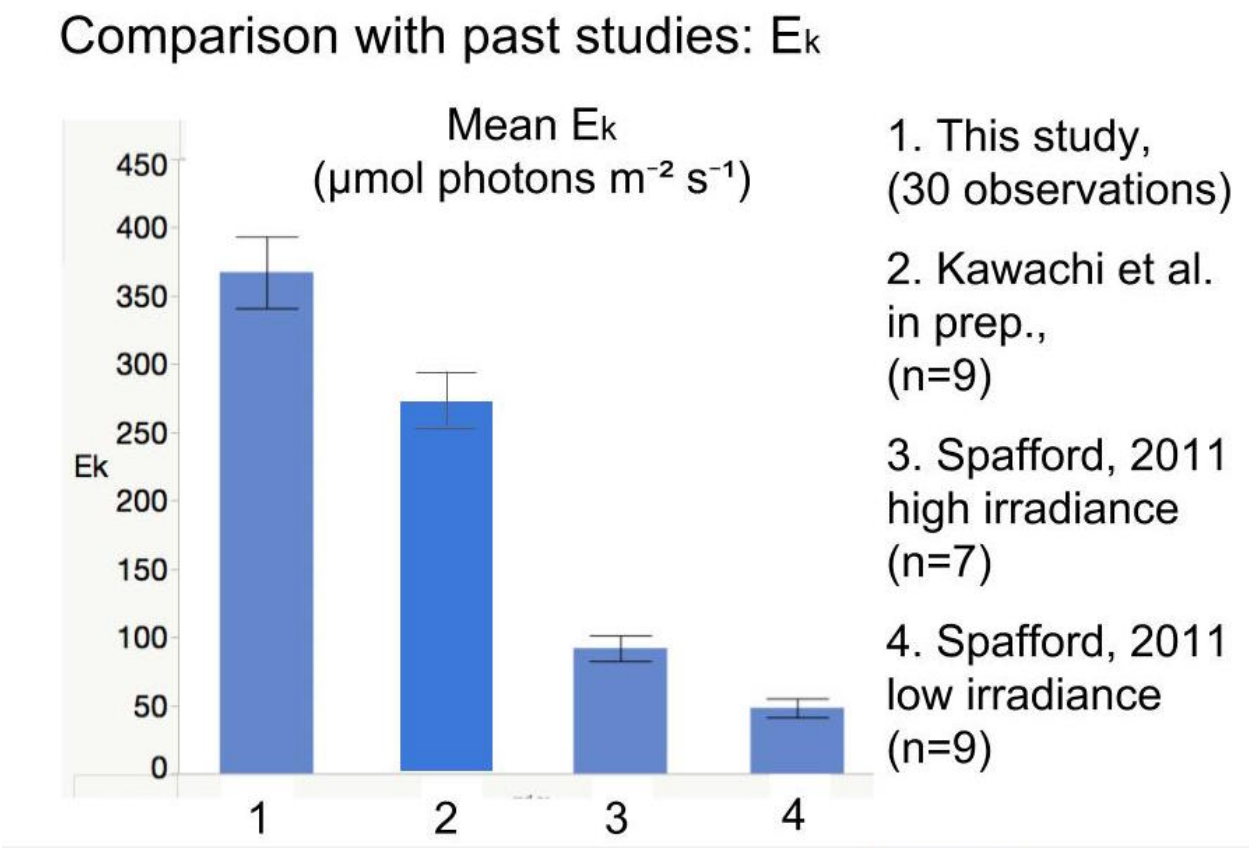

Figure 2.7: Comparison of E_k values. Means and standard errors shown are from this study, Kawachi *et al.*, in prep and Spafford (2011).

Appendix A: pH Measurements

pH measurements were run spectrophotometrically using metacresol purple (mCP) indicator dye according to Standard Operating Procedure (SOP) 6b (Dickson, Sabine & Christian, 2007). Spectrophotometry measurements from the first trial were conducted using a computer assisted HP Diode Array, Botany, University of Hawai‘i at Mānoa. Spectrophotometery measurements for subsequent trials were conducted using a Molecular Devices SpectraMax M2 (CA, USA) spectrophotometer and Softmax software located at the Hawai‘i Institute of Marine Biology (HIMB).

Mixing of the dye: Metacresol purple tablets (Acrōs Organics, Thermo Fisher Scientific, Belgium) 76.49 g of metacresol purple (mCP) tablets were mixed with 100 mL deionized water to produce a 2 mM solution. The dye was then mixed with dilute $NaOH_{(aq)}$ to bring the pH of the dye close to 7.9 (Dr. Meter pH-100)

Calibration of the dye: The pH of dye differs slightly from the pH of each of sample, therefore addition of the indicator dye changes the pH of each sample slightly. To account for this, each batch of mCP dye must be calibrated. Beakers containing 50 mL of filtered seawater were prepared across a spectrum of pH values between 8.5 and 7.7, using CO_2 and dilute $NaOH_{(aq)}$ to alter the pH values. The approximate pH values of each prepared seawater solution were 8.3, 8.1, 8.0, 7.9, and 7.8.

For each seawater solution sample, the following steps were taken.

1. Using a water bath, samples were maintained at 25°C during the spectrophotometry procedure.
2. The spectrophotometric analysis software (Softmax Pro) was set to analyze absorbance values at wavelengths 578 and 434, with a reference wavelength at 730.

3. A cuvette and pipette were rinsed with sample seawater twice. 3 mL of sample seawater were pipetted into the cuvette.
4. The cuvette exterior was wiped thoroughly with a tissue (Kimwipe) to remove excess oil and dust.
5. The cuvette was placed into the spectrophotometer (Molecular Devices SpectraMax M2, CA, USA) and analyzed as a blank sample.
6. The cuvette was removed from the spectrophotometric machine and 50 μL of mCP indicator dye was added to the cuvette. The cuvette was covered and slowly inverted to ensure proper mixing.
7. The cuvette was wiped thoroughly with a tissue.
8. The cuvette was placed into the spectrophotometer for analysis of absorbance values.
9. For dye calibration purposes, steps 6-9 were repeated once with the same sample of water in the same cuvette.
10. All absorbance values and blank values were recorded.*
11. All absorbance values (first addition of dye and second addition of dye) were corrected using Eqs. 1 & 2.

* Some spectrophotometer software automatically corrects absorbances using the blank samples and reference wavelengths. In this case, only absorbance values of wavelengths 578 and 434 must be recorded, and Eqs. 1 and 2 below may be ignored.

Using this protocol, a regression line was created from the ratio of the absorbance values after the first addition of dye, and the difference between the ratio of the absorbance values after the second addition of dye (Eq. 3). This regression allowed an approximation of the change in the pH resulting from the addition of dye so that compensation can be made (Clayton & Bryne, 1993 *in* Dickson, Sabine & Christian, 2007).

Equations 1 & 2: Calculating corrected absorbance values

$corr_{578} = (raw_{578} - blank_{578}) - (raw_{730} - blank_{730})$
$corr_{434} = (raw_{434} - blank_{434}) - (raw_{730} - blank_{730})$

$blank_{578}$, $blank_{434}$, and $blank_{730}$ are the absorbance values obtained from spectrophotometric analysis of a blank/undyed sample.
raw_{578}, raw_{434}, raw_{730} are the raw absorbance values obtained from spectrophotometric analysis of a dyed sample.
$corr_{578}$, $corr_{434}$ are the absorbance values corrected using blank and reference absorbances

Equation 3: Equation for the calibration linear regression

$$\Delta(A_{578}/A_{434}) = a + b\ (A_{578}/A_{434})$$

A_{578} and A_{434} are the corrected absorbance values at wavelengths 578 and 434, respectively.
$\Delta(A_{578}/A_{434})$ is the change in the absorbance ratio between the first addition of dye and the second addition of dye.
a and *b* are the intercept and slope specific to the calibrated dye batch and are used to correct for the pH changes resulting from adding dye to the seawater sample.

Measuring the pH of a sample: Measuring the pH of a sample is similar to calibrating the dye batch. The same procedure, excluding step 10, was used to measure the absorbances of each sample. The ratio of A_{578}/A_{434} was then calculated from these values and corrected for the dye measurement using the slope and intercept values from the calibration procedure (Eq. 4). A pH value was then calculated from the corrected absorbance ratios (Eq. 5). Temperature and salinity measurements of the sample were also necessary for these calculations.

Equation 4: Calculating the corrected absorbance values

$$(A_{578} / A_{434})_{corr} = (A_{578} / A_{434}) - [a + b(A_{578} / A_{434})]$$

A_{578} and A_{434} are the sample's measured absorbance values at wavelengths 578 and 434, respectively.

$(A_{578} / A_{434})_{corr}$ is the corrected ratio, which takes into account the pH change caused by the addition of indicator dye. This is the ratio used for the seawater sample pH determination calculations.

Equation 5: Calculating the pH of the seawater sample

$$\mathrm{pH} = \mathrm{p}K_2 + \log_{10}\left[\frac{(A_{578}/A_{434})_{corr} - 0.00691}{2.222 - 0.133(A_{578}/A_{434})_{corr}}\right]$$

$$\mathrm{p}K_2 = \frac{1245.69}{T/\mathrm{K}} + 3.8275 + 0.00211(35 - S)$$

T is temperature at the time of spectrophotometry measurement (estimated here at 298.15K).
K is the unit Kelvin, making the final value unitless.
S is salinity of the sample in parts per thousand (estimated here at 34.5 ppt)

Literature Cited

Clayton TD, Byrne RH. 1993. Spectrophotometric seawater pH measurements: total hydrogen ion concentration scale calibration of m-cresol purple and at-sea results. *Deep Sea Research Part I: Oceanographic Research Papers* 40:2115-2129.

Dickson AG, Sabine CL, and Christian JR, eds. 2007. Guide to best practices for ocean CO_2 measurements. *PICES Special Publication* 3:1-191.

Appendix B: Alkalinity Titrations

Alkalinity titrations were done using an automatic titrator (Titrino Plus 877, Metrohm) with a pH glass electrode (9101 Herisau, Metrohm) and 0.1 N HCl . The procedure for alkalinity titrations without an automatic titrator is outlined in Dickson, Sabine & Christian (2007) (SOP 3b: Determination of total alkalinity in seawater using an open cell titration).

Calibrating the pH electrode: Prior to each use, the titrator's pH electrode was calibrated. The pH electrode was rinsed with deionized water and gently dried with a tissue (Kimwipe) before and between calibrations. NBS buffer solutions of pH 4.00 ± 0.01 (YSI 3821) and 7.00 ± 0.01 (Oaklon) were used during calibration. After the calibration, a slope percentage is given which indicates the level of functionality of the pH electrode. For this experiment, all slope values were above 98%.

Preparing the automatic titrator: Prior to each use, a preparation sequence was run on the titrator. This preparation sequence pumps acid through the tubing system to remove any bubbles that may have become trapped in the tubes. The software runs the preparation function automatically with user prompting. For this experiment, the burette tip was placed into a waste beaker of seawater and during the prep function, tubes were gently tapped to facilitate the removal of air bubbles.

Titrating a seawater sample: The automatic titrator system incorporates a pH electrode, a thermometer, and a burette for dispensing acid. The pH electrode reads the pH of the solution indicating when an inflection point is reached. The thermometer is used to correct for variance caused by temperature. The burette tip automatically dispenses dilute HCl acid at intervals to detect the volume of acid needed to neutralize the alkalinity in the sample, and the titrator software calculates TA from this value. Included in the set-up is an air pump attached to a length

of thin tubing. During the titration, carbon dioxide evolves in solution and the air pump helps to purge this gas from the sample after the first inflection point. All of these components were rinsed with filtered seawater and dried gently with a tissue before and between sample measurements.

1. All bottles containing sample seawater were kept in a water bath at 25°C (293.15 K) prior to titration to stabilize temperatures.
2. Approximately 100 mL of seawater was placed into a clean beaker using an automatic pipettor.
3. Seawater was added or removed from the beaker using a plastic dropper pipette until the seawater weighed between 100.000-100.100 g using a balance with ±0.001 g precision (Mettler Toledo XS403S). Weights were inputted into the automatic titrator software to assist in calculations.
4. The pH electrode, thermometer, and burette were all submerged in the sample beaker and a holder was used to maintain their position.
5. A magnetic stir bar was inserted into the beaker and the user prompted the automatic program to begin a titration.
6. The start time of each titration, sample weight, and sample temperature were manually recorded.
7. After the first pH inflection point was reached (displayed on the titrator monitor), air was added to the sample.
8. At the end of the titration, the pH and total alkalinity were manuallly recorded.

Maintaining accuracy assurance using certified reference materials (CRM): Every two weeks, a CRM was run to check the accuracy of the titrator using the same procedure requried for typical seawater samples as detailed above.

Literature Cited

Dickson AG, Sabine CL, and Christian JR, eds. 2007. Guide to best practices for ocean CO_2 measurements. *PICES Special Publication* 3:1-191.

Appendix C: HOBO observations (lux) to PAR (µmol photons m^{-2} s^{-1}) conversion

HOBO Pendant Data Loggers (Onset, MA, USA) referred to as "HOBOs" were placed close to the algal tissue in each aquarium to take light and temperature measurements every 30 m. A single LI-COR Sperical Underwater Quantum Sensor (Lincoln, NE, USA) was also deployed in the water bath, adjacent to two aquaria, at the same depth as the algal samples. The sensor was attached to a data logger (LI-1400, LI-COR, Lincoln, NE, USA) which was programmed to measure light every 30 min between 0630-1900. The HOBO measures light in lux across a wide spectrum (~150-1200 nm wavelength) and measures only light recieved at 90° (cosine sensor). The LI-COR sensor is calibrated to measure Photosynthetically Active Radiation (PAR), which is light in the 400-700 nm wavelength range, coming in from all angles (360° or 4π sensor). In biology, PAR is often measured in µmol photons m^{-2} s^{-1} and is sometimes referred to as Photosynthetic Photon Flux Density (PPFD) (Björn, 1994). This is a quantum light unit, meaning that it measures the quanta of photons recieved per unit area per unit time, and is more applicable to photosynthetic studies due to the quantized nature of photosynthetic chemistry (Thimijan & Heins, 1982; Bolton & Hall, 1991). Many references give a simple linear conversion equation for converting sunlight from lux to PAR (Thimijan & Heins, 1982). However, the ratio of total radiation to PAR is affected by many factors, such as location, season, and sky conditions (Jacovides *et al.*, 2004). Additionally, in this case we are not only trying to convert between units, but also trying to convert a planar measurement to a 3D measurement. For this reason, we decided to look at the correlation between the HOBO measurements and the LI-COR measurements to create our own conversion equation.

To create a place-specific conversion of lux to PPFD, we looked at all light measurements between the hours of 0630-1900. We averaged the measurements taken by the

HOBOs in the two tanks closest to the LI-COR sensor. The LI-COR sensor only recorded data from two trials, due to researcher error. We paired the averaged HOBO measurements from those two trials with the LI-COR data and matched the time-stamps of each observation. A graph of LI-COR versus averaged HOBO data was created (Figure C.1). After visual inspection, it appeared that light values from the morning (0630-1130) and light values from the afternoon (1400-1900) followed two distinct regression curves, while light values from midday (1200-1330) showed no pattern at all (Figure C.2). After log-transforming both variables to create a more linear relationship, a linear regression was performed on morning, noon, and afternoon data sets. The linear regression fitted the morning and afternoon data very well (Table C.1), but did not fit the noon data. Since no clear pattern could be found for the noon data, HOBO values before 1230 were clumped into morning and values at or above 1230 were clumped into afternoon. Using the two equations, all individual HOBO data was converted from lux values measured with a cosine meter to the analogous μmol photons m^{-2} s^{-1} measured with a 4π sensor.

Literature Cited

Björn LO. 2014. Principles and nomenclature for the quantification of light. In *Photobiology* 21-25. Springer New York.

Bolton JR, Hall DO. 1991. The maximum efficiency of photosynthesis. *Photochemistry and Photobiology* 53(4):545-548.

Jacovides CP, Timvios FS, Papaioannou G, Asimakopoulos DN, Theofilou CM. 2004. Ratio of PAR to broadband solar radiation measured in Cyprus. *Agricultural and Forest Meteorology* 121(3):135-140. DOI: 10.1016/j.agrformet.2003.10.001

Thimijan RW, Heins RD. 1983. Photometric, radiometric, and quantum light units of measure: a review of procedures for interconversion. *HortScience* 18(6):818-822.

Table C.1: Summary of linear regressions for log-transformed LI-COR values and log-transformed averaged HOBO values.

Time of observations	R^2 value	P value	Equation
Morning	0.91	<0.0001	Log (LI-COR) = -1.231 + 0.723 Log(HOBO)
Noon	0.00	.927	Log (LI-COR) = 4.219 + 0.0131 Log(HOBO)
Afternoon	0.84	<0.0001	Log(LI-COR) = -0.819 + 0.564 Log(HOBO)

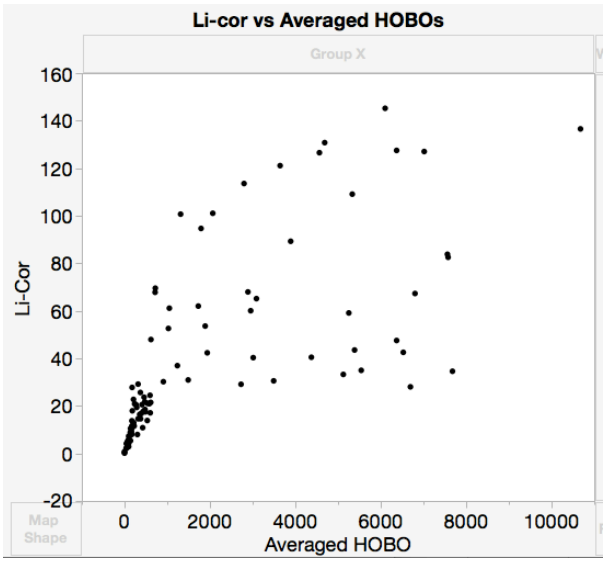

Figure C.1: LI-COR versus HOBO data over two trial periods.

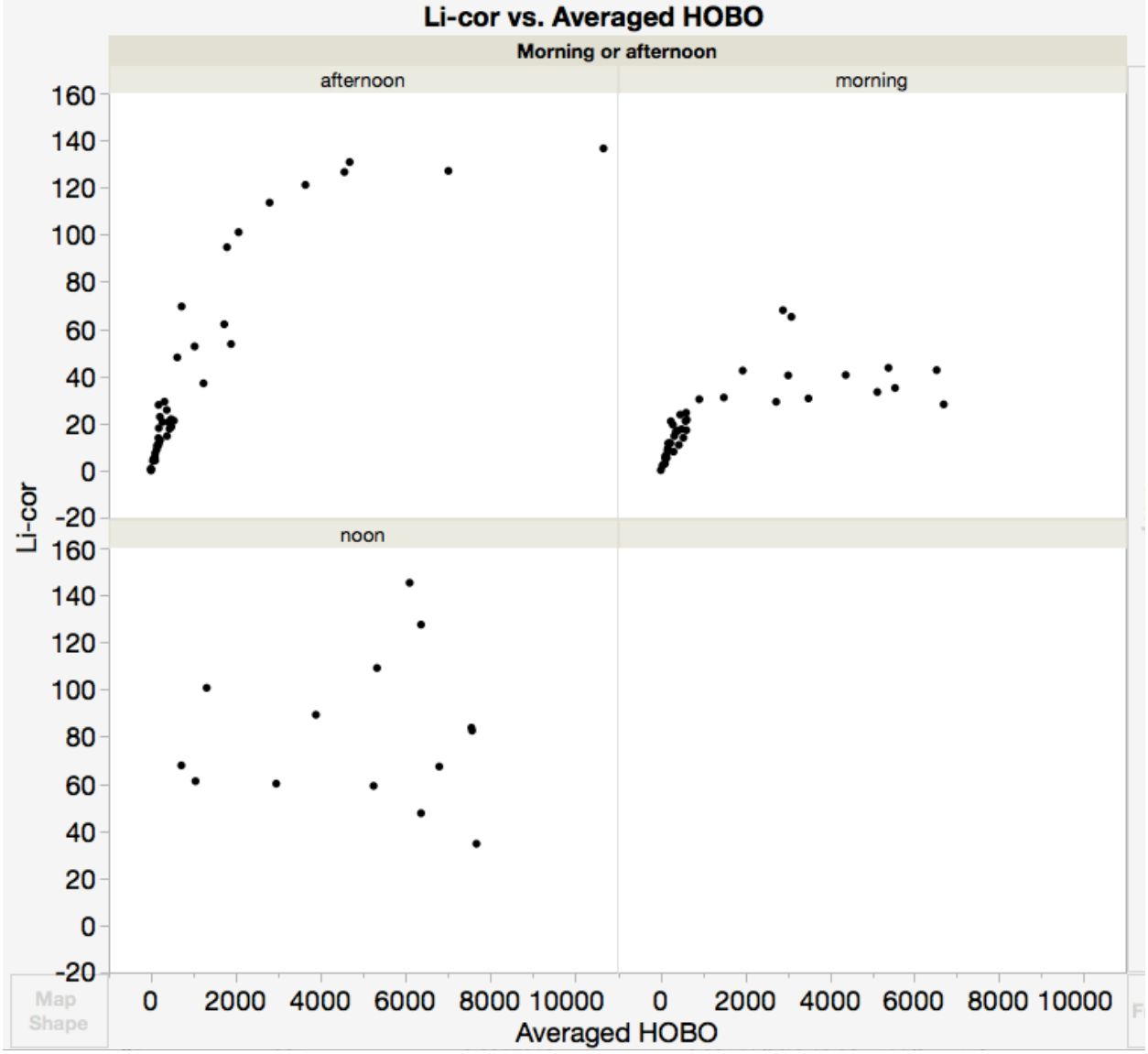

Figure C.2: LI-COR versus HOBO data separated by time of observation (morning 0630-1130, midday 1200-1330, or afternoon 1400-1900).